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Almqvist & Wiksell, Uppsala 1974

Despite the potentially serious consequences of A V no experimental study of the phenomenon in man has been published so far The actual mechanism has been a matter of dispute for years, and opinions differ as to how pressure changes might cause vertigo Most authors agree that a pressure difference between the middle ear cavity and the atmosphere in some way causes the vertigo It is also generally assumed that the vertigo is caused by a relative overpressure in the middle ear since most subjects who have some experience of A V have observed that it occurs when the middle ear pressure has been increased indirectly by lowering the ambient pressure at ascents or directly by blowing against a clamped nose (Valsalva's manoeuvre)

The mechanism by which pressure affects the inner ear is, however, still unknown Vestibular stimulation due to pressure changes in the middle ear was early observed in subjects with a labyrinthine fistula In 1908 Bárány introduced the "fistula test", by which such fistulas could be diagnosed by compression and aspiration in the external ear canal in subjects having an eardrum perforation due to chronic otitis media In 1919 Karlefors & Nylen (Nylen, 1923) introduced the term "pseudo-fistula symptom" for the vertigo ich could be elicited in some subjects although labyrinthine fistula could be found The on was, however, noticed long before by many authors who had observed "fistula symptoms without fistula" These symptoms were often seen in subjects with lues and explained as due to an abnormal mobility of the stapes footplate caused by luetic periosteitis around the stapes That displacement of the stapes footplate could cause vestibular stimulation was also noted by House (1968) when pushing down the stapes with a needle during ear surgery As for the mechanism through which pressure changes may induce vertigo in normal subjects it has been suggested that pressure somehow induces an unusual large displacement of the stapes footplate, and thus causes inner ear fluid to flow and stimulate the vestibular receptors (Fields, 1958, Heller, 1960, Hilger, 1964) Some authors consider the mechanism by vertigo is elicited akin to the fistula mecha Thus Melvill Jones (1957) and Benson (suggest that the bony wall separating the m ear cavity from the horizontal canal may thin in certain subjects as to become defc when the middle ear pressure differs fro ambient pressure

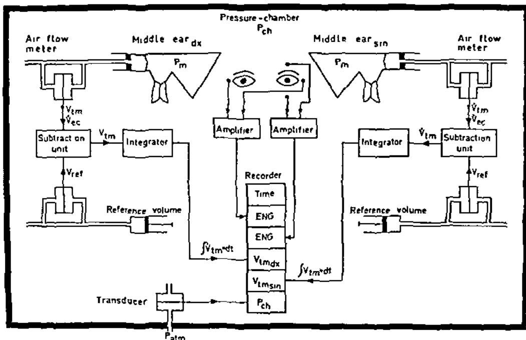
Furthermore, the observations of vertig der field conditions appear frequently to b long (several minutes) to be caused by n ments of the stapes (Lundgren, 1965, V marti & Bradley, 1970, Lundgren et al, 1 However, from these and other studies it ap that A V most frequently occurs during asc both in flight and diving, i e by a fall in bient pressure with a resultant relative pressure in the middle ears This overpre seems to be crucial for eliciting A V The no information in the literature about the of overpressure necessary for eliciting ve but it is well known that most pilots and d at ascents usually do not clear their ears acti but wait for their ears to be cleared pass when the relative overpressure has incre enough to force the Eustachian tubes o According to this it seems to be of esse interest to find out whether the forcing pres is strong enough to cause vertigo in cer subjects or if other mechanisms are involve the phenomenon

The aims of the present work were (i) to termine the level of relative overpressure in middle ears that might result from amb pressure decrease, i e the level of overpress necessary to force the Eustachian tubes o (forcing pressure), (ii) to see if such overpress can cause A V in normal subjects, and (iii) investigate, by means of ENG, if experiment elicited A V might be of vestibular origin

EQUIPMENT AND METHOD

The following symbols are used

Patm, atmosphere on ground
Pm, pressure in the middle ear
Ptm, pressure gradient across the eardrum



Outline of the equipment used for recordings of isplacement of the eardrums (*Vtm*) at changing nt pressure (*Pch*) For details, see text

pressure in the chamber, i e. ambient pressure

pressure in the external ear canal

volume of the airfilled middle ear space

volume displacement of the eardrum in relation to its neutral position

c, volume of the mucous membrane lining the middle ear space

airflow through the resistor of the ear canal flowmeter caused by the volume displacement of the eardrum

airflow through the resistor of the ear canal flowmeter, caused by expansion or compression of the gas volume in the external ear canal and in the flowmeter system by changing the ambient pressure

, airflow through the resistor of the reference flowmeter, caused by expansion or compression of the gas volume in the reference system by changing the ambient pressure

Δ before the symbol indicates a change of the variable. Pressure is expressed in cm H_2O, volume in microlitre, µl, or millilitre, ml, and airflow in microlitre/sec, µl/sec

Pm, Pch and Pec are relative to atmosphere pressure on the ground

Pm_d middle ear pressure minus the pressure of saturated water vapour at 37°C, this being regarded as non-compressible in connection with Boyle's law

Kmuc, *volume-pressure relation of the middle* ear mucosa (compliance), at the experimental pressure conditions of the present study

Fig 1 gives an outline of the equipment used for the recordings A pressure chamber was used in which it is possible to simulate "ascents" and "descents" of 90 cm H_2O in 25 sec, with a constant rate of pressure change during 90% of the time The subjects were placed in the

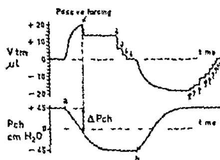


Fig 2 Recording of eardrum volume displacement (Vtm in μl) and chamber pressure (*Pch in* cm H_2O) Eardrum movement outwards (+) and inwards (−) Each examination starts with a simulated descent with a pressure increase of +45 cm H_2O It is then checked that the eardrum is in its neutral position (Vtm ≈ 0) after active equilibration The simulated ascent then starts (*a*) with a pressure decrease of 90 cm H_2O The Eustachian tube is opened passively (passive forcing), and the eardrum moves inwards to the neutral position when the middle ear pressure is equilibrated to the chamber pressure by deglutitions (*arrows*) When the chamber pressure is increased (*b*) the eardrum moves inwards, and when the middle ear pressure is equilibrated by deglutitions (*arrows*) the eardrum again moves to its neutral position

chamber and exposed to pressure changes A polyethylene catheter with its end through a rubber disc was inserted *into* the inner bony part of the external ear canal connecting the space between the eardrum and the rubber disc with the measuring device A method was used, de-vised by Ingelstedt et al (1967) and Elner et al (1971a), by which it is possible to record the displacement of the eardrum (Vtm) in relation to its neutral position when the ambient pressure (Pch) is changed The method implies free air communication between the outside of the eardrum and the ambient pressure and is based on recording, by means of a flowmeter, the airflow caused by the eardrum displacement (Vtm) The flow Vec must be eliminated for an isolated recording of Vtm during ambient pressure changes This elimination is possible by using an identical flowmeter system with an adjustable reference volume *After* this elimination the flow signal (Vtm) is integrated and Vtm is recorded From these recordings of Vtm and Pch it is possible to determine the ambient pressure change (ΔPch) necessary to cause a passive opening of the Eustachian tubes (passive forcing) Fig 2 gives an example of a recording For details of the method and the equipment see Ingelstedt et al (1967) and Elner et al (1971)

Simultaneously with the recordings of Pch and Vtm, recording of eye-movements by means of electronystagmography (ENG) was performed [illegible] of experiments eye-movements [illegible] and performed [illegible] ject's eyes open in order to get a reliable [illegible] of any vestibularly induced eye-movement (Tjernström, 1973) In the ensuing series of experiments only horizontal leads were used, as vertical nystagmus did not appear in the first series Specially devised electrodes for d c -recording were used (Siemens-Elema AB, Sweden)

Calibration of the equipment for recording Vtm and Pch was performed before and after every test according to the original method Eye movements were calibrated before and after every test for 30° *in* each direction

Estimation of the forcing pressure as equivalent to ΔPch (the pressure change in the chamber during the ascent recorded at the moment of passive opening of the tube) is only approximate For a correct determination of the pressure gradient across the tube, i e the pressure gradient across the eardrum (Ptm) necessary *to* force the tube open passively, knowledge is required of the middle ear pressure (Pm) as well as of the ambient pressure (Pch) at the moment of passive opening Before each test it was checked that the eardrum was *in* its neutral position which means that Pch = Pm, i e Ptm = 0 At the moment of passive forcing the pressure gradient across the eardrum (Ptm) is equal to the difference between the pressure change in the chamber (ΔPch) and the pressure change in the middle ear (ΔPm), i e

$$Ptm = \Delta Pch - \Delta Pm \qquad \text{(eq 1)}$$

Fig 3 gives an outline of the different variables involved Determination of the middle ear pressure change (ΔPm) during changing ambient pressure (ΔPch) requires knowledge of the following factors

ig 3 Middle ear model with its variables For explana ion of symbols see text

m, ΔVtm and ΔVmuc

Since changes in the middle ear pressure cause change in the volume of the vessels lining the panic cavity, and thus affect the middle ear ressure, this volume factor (Vmuc) has to be etermined

When these factors are known it is possible to alculate the middle ear pressure change (ΔPm) at changing ambient pressure by using the fol lowing equation derived from Boyle's law (Ingel stedt et al , 1967)

$$\Delta Pm \quad \frac{\Delta Pch \times Kmuc + \Delta Vtm}{Kmuc + \frac{Vm}{Pm_a}} \qquad \text{(eq 2)}$$

Kmuc = the volume pressure relation (compli ance) of the middle ear mucosa (see text)

The volume of the middle ear may be determined directly via a perforation of the eardrum (Flisberg et al , 1963, Andreasson 1973), or indirectly across an intact eardrum (Elner et al , 1971*a*) It has been shown that there is a linear relation between a roentgenological planimetri cal determination of the area (Am) and a direct volume determination (Vm) of the airfilled middle ear system (Flisberg & Zsigmond, 1965, Andreasson, 1973) According to Andreasson this relation was Vm = 0 49 × Am − 0 38 in a study of 9 subjects with a traumatic perforation of the eardrum

In the present study, the middle ear volume was determined indirectly by measuring the area of the ear cell system on X ray films (projection II according to Runstrom 1933), applying a planimeter method similar to that introduced by Diamant (1940) This is the same method as that used by Flisberg & Zsigmond (1965) and An dréasson (1973)

The volume displacement of the eardrum was determined by the method devised by Ingelstedt et al (1967) and Elner et al (1971*a*)

The volume variation of the mucosa (ΔVmuc) has been studied by Andreasson et al (1974), and the volume pressure relation (compliance, Kmuc) was calculated at 0 5 μl/cm H_2O in the ex perimental conditions used in the present study

The diffusion of gas from the middle ear (ΔVdiff) also affects the middle ear pressure Since the diffusion is slow (0 5–1 0 μl/min, Elner, 1971, Ingelstedt & Jonson, 1967) this factor is negligible in studies on rapid events as in the present study with time periods of only about 20 sec

MATERIAL

The material consisted of 79 subjects aged be tween 21–50 years, 51 of whom men and 28 women All subjects were regarded as otologic ally normal with no history of ear disease and a normal ear examination The hearing threshold was within 0–20 dB (related to ISO standard, 1964) All subjects were free from signs of catar rhal infection at nose and throat examination and there was no history of labyrinthine disease

EXPERIMENTAL PROCEDURE

A *Passive equilibration at simulated ascents—monaural recording*

In this part of the present study recordings were made only from one ear in each examination as there was no equipment for binaural recording The forcing pressure was defined as the level of the chamber pressure change at the moment of passive forcing The middle ear pressure (Pm) was not calculated

(1) All subjects (79) were examined in the seated position and the Vtm was recorded during simulated ascents from +45 to −45 cm H_2O in 25 sec The subjects were instructed to avoid active equilibration of the middle ear pressure during each ascent

(2) Re-examinations at intervals of several

months were made on 14 subjects (18 ears) in order to assess the individual variation of the forcing pressure from one time to another The examinations were performed in the same way as described above

B Active and passive equilibration at simulated ascents and descents—binaural recordings and simultaneous ENG-recording of eye-movements

In this part of the present study recordings were made from both ears simultaneously Ptm at the moment of passive forcing was calculated Eight subjects were examined in the seated position and Vtm was recorded from both sides together with ENG The subjects were instructed to keep their eyes open in total darkness, to look straight ahead and to avoid blinking During the tests with active equilibration the subjects were told to swallow as often as possible and during the tests with passive equilibration they were instructed to avoid active regulation of the middle ear pressure

RESULTS

A Monaural recording of passive equilibration at simulated ascents

1 The tube was forced open in all subjects when exposed to simulated ascents The forcing of the was caused by overpressure in the middle ue to the pressure decrease in the chamber

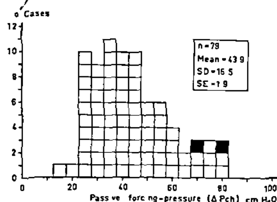


Fig 4 Distribution of 79 otologically healthy subjects in relation to passive forcing pressure (*abscissa*) The forcing pressure is given as the pressure change in the chamber (*ΔPch*) necessary to force the Eustachian tubes open Black squares indicate two subjects who reported vertigo during the examination

Table I *Statistical evaluation of the individual variation of the passive forcing pressure recorded on 14 subjects (18 ears) at different sessions and with different numbers of examinations*

$\bar{x}$ = a erage of all tests on [illegible]

Ears	No of sessions	No of examinations	Passive forcing pressure (ΔPch) cm H_2O x	$d(x)$
1	4	9	67 2	2 6
2	2	4	43 3	3 8
3	2	4	69 8	3 8
4	3	14	75 1	2.7
5	2	14	49 6	3 3
6	2	11	82 3	3 4
7	3	15	71 3	2 8
8	2	13	51 1	3 3
9	3	17	68 1	2 7
10	2	5	31 3	3 7
11	2	5	49 6	3 5
12	3	9	46	2 8
13	5	21	36	2 3
14	4	9	46 3	2 6
15	3	7	39	2 9
16	4	14	77 9	2 5
17	2	12	47 4	3 3
18	6	26	31 8	2 0

The forcing occurred at different pressure levels in different subjects The distribution of the subjects as regards the forcing of the tubes in relation to the pressure decrease in the chamber is given in Fig 4 Two out of 79 subjects reported a brief period of vertigo during the examination and these 2 subjects are indicated in Fig 4 by black squares It was possible to reproduce the vertigo in these 2 subjects when re examined months later, and simultaneous ENG revealed horizontal nystagmus indicating that the vertigo was of vestibular origin Vertical nystagmus was never seen

2 In Table I the results of the re examinations of 18 ears are presented The passive forcing pressure is given as the ambient pressure change (ΔPch) at the moment of passive forcing of the tubes The Table presents a statistical evaluation of the results of the examinations at different sessions The variation of the forcing pressure

each subject was low despite intervals of veral months between the different sessions

omment As seen from Fig 4, the tubes of the vo subjects with vertigo were not forced open ntil there was a great pressure decrease in the hamber indicating that the forcing pressure in iese subjects was high As however the recordgs were made only from one side, it has so ar been impossible to find out from which ear he vertigo was elicited These two subjects were e examined with pressure recordings from both ides simultaneously together with ENG

Binaural recording of passive and active equibration during simulated ascents and descents

e two subjects reported vertigo each time when xposed to ascents and passive clearing of the ars Horizontal nystagmus coincided with every eriod of vertigo In each subject the left and he right tube were forced open at different hamber pressure levels, indicating different orcing pressure for the left and the right tube

In Table II is presented the magnitude of the ressure decrease in the chamber (ΔPch) at vhich the tubes were forced open, recorded at ifferent examinations (The Table also includes he monaural recording of these two subjects) Nystagmus intensity, expressed as the maximal velocity of the slow nystagmus phase and reports of vertigo are also indicated No nystagmus during ascents with active equilibration was seen and no vertigo was reported Descents with and without active equilibration caused neither vertigo nor nystagmus, and there was no passive clearing when the subjects were exposed to descents

Both subjects could equilibrate static as well as dynamic over and under pressures in a normal way, and belonged to tubal function groups IB and IC (very good tubal function) according to the tubal function groups presented by Elner et al (1971*b*)

Fig 5 shows a recording at a simulated ascent with passive clearing of the ears The left tube was forced open when the ambient pressure was lowered by 49 5 cm H_2O (*a*), and the right tube when lowered by about 81 cm H_2O (*b*) When the ambient pressure was lowered by about 66 cm H_2O an eye-deviation started, directed to the left (*c*), and was followed by nystagmus to the right The right beating nystagmus continued until the right tube was forced open, after which it decreased and changed direction, and a left-beating nystagmus was recorded for about 20–30 sec Just before the right tube was forced open the subject reported vertigo

Comment A very interesting feature of this recording is that the vestibular stimulation was not induced at the moment of pressure regula-

Table II *Chamber pressure decrease (ΔPch) at which the Eustachian tubes were forced open as recorded at different sessions with different numbers of examinations*

The Table also includes the monaural recordings of the two vertiginous subjects Nystagmus intensity and reports of vertigo as indicated

Session no	Passive forcing cm H_2O ΔPch_{dx}	ΔPch_{sin}	Vertigo	Max. velocity of eye-deviation in slow phase, °/sec
Subject I				
1	81	Not recorded	+	Not recorded
2	79 5	Not recorded	+	3
	82.5	Not recorded	+	3
3	81	49.5	+	4 5
	87	48	+	3
	78	48	+	5
	79.5	46 5	+	5
	81	46 5	+	3 5
Subject II				
1	72.5	Not recorded	+	Not recorded
	67 5	Not recorded	+	Not recorded
2	76 5	Not recorded	+	2
	72	Not recorded	+	3 5
3	64 5	46 5	+	1
	69	39	+	1
	60 5	43 5	−	0 5
4	64 5	43 5	−	2.5

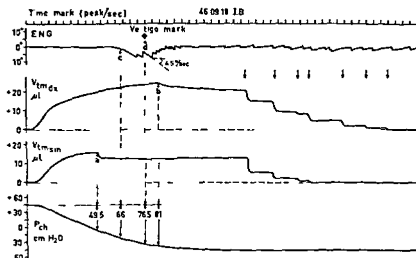


Fig 5 Electronystagmogram (E… volume displacements of … (Vtm$_{dx}$) and left (Vtm$_{sin}$ in … eardrums and chamber pres… (Pch in cm H_2O) during pas… forcing of the Eustachian tu… Arrows mark deglutitions. Pa… clearing of left ear (*a*) and … ear (*b*) Nystagmus starts when … ambient pressure is changed by … cm H_2O (*c*)—slow nystagmus ph… The subject reports vertigo … when the chamber pressure … lowered by 76 5 cm H_2O … further comments see text

tion, but during the period of asymmetric middle ear pressure and not until the pressure in the not-cleared ear had reached a certain level To assess the asymmetry and to find out the real difference between the middle ear pressure and the ambient pressure, i e the pressure gradient across the eardrum (Ptm) it was necessary to calculate the middle ear pressure on both sides at the moment of passive forcing (see eqs 1 and 2)

ΔPch and ΔVtm were calculated directly from the recordings The middle ear volumes were … by the planimeter method and trans… from area (Am) to volume (Vm) by using correction fractors determined by Andreas (1973) The results are given in Table III

In the material presented by Andréasson (1973) there were no normal subjects but only subjects with a traumatic perforation of the ear drum As however the figures of the area and the volume of these subjects fall within the limits of normal values as described by other authors (Diamant, 1940, Ingelstedt et al, 1967, Elner, 1971), it was assumed that the figures are v… also for the normal subjects in the present st… In order to verify this assumption the middle … volume on one side of 2 subjects (subject … and IV) was determined both indirectly acc… ing to Ingelstedt et al (1967) and Elner et …

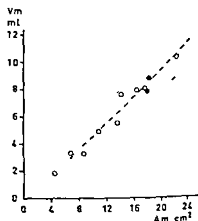


Fig 6 Comparison between area (*Am*) in cm² and vol… (*Vm*) in ml (Andreasson 1973) ○ =9 subjects wi… traumatic perforation of the eardrum The area … determined by a roentgenological planimetrical met… (Diamant 1940 Flisberg & Zsigmond 1965 Andréass… 1973) and the volume by a direct volume determina… of the airfilled middle ear system (Flisberg & Zsigmo… 1965 Andréasson 1973) ● 2 subjects with a nor… ear examination The area was determined with the sa… roentgenological planimetrical method as mentio… above and the volume by an indirect determina… according to Ingelstedt et al (1967) and Elner et … (1971*a*) Determinations were made from the right … of both subjects The lines of short dashes (curv… indicate 95% confidence belt for a predicted "y" (S… decor & Cochran 1973*a*)

Table III *Area in cm² and volume in ml of the middle ear cell system*

	Dx		Sin	
Subject	Am	Vm	Am	Vm
I	21 4	10 9	19 4	9 1
II	15 3	7 1	13.2	6 1

(1971*a*) and by the planimeter method, and it was seen that these two different volume determinations gave very similar results (Fig 6) The results of the calculation of the pressure gradient across the eardrum (Ptm) at the moment of passive forcing of the tubes are given in Table IV

Comment As seen from Fig 4, there were however another 7 subjects with a high forcing pressure (ΔPch > 65 cm H_2O), none of whom had complained of vertigo Out of these 7 subjects 4 were re-examined with binaural recordings and ENG, and 2 subjects with a forcing pressure according to the mean value ('normal subjects") were also included

Three out of 4 subjects with high forcing pressures reported vertigo at one occasion each when re-examined (subject VI, VII and VIII) Asymmetric forcing pressures were also seen in

Table IV *Chamber pressure decrease (ΔPch) and pressure gradient across the eardrum (Ptm) at the moment of passive opening of the Eustachian tubes recorded at different sessions and with different numbers of examinations*

The Table also includes the monaural recordings of the two vertiginous subjects

Session no	Passive forcing cm H_2O			
	ΔPch_{dx}	Ptm_{dx}	ΔPch_{sin}	Ptm_{sin}
Subject I				
1	81	75 9	Not recorded	
2	79 5	74 4	Not recorded	
	82 5	77 4		
	81	75 4	49 5	45 4
	87	81 1	48	44
3	78	72 6	48	44
	79 5	74	46 5	42 5
	81	75 4	46 5	42 5
Subject II				
1	72 5	65 7	Not recorded	
	67 5	61		
2	76 5	68 5	Not recorded	
	72	64 9		
3	64 5	58 2	46 5	40 7
	69	62 4	39	33 9
	60 5	54 6	43 5	38 2
	64 5	57 7	43 5	38 2

Table V. *Area in cm² and volume in ml of the middle ear cell system*

Subject	Dx		Sin	
	Am	Vm	Am	Vm
III	18 3	8 6	22.2	10 6
IV	17 8	8 3	17 8	8 3
V	23 8	11 2	25 8	12 3
VI	21 5	10 5	22 9	10 8
VII	25 5	12.4	23 8	11 6
VIII	17 0	7 8	17 3	8 1

these subjects Horizontal nystagmus was recorded at every test in which vertigo was reported However, nystagmus was also seen in some of the tests though the subjects did not notice any vestibular stimulation When the 2 subjects with a normal forcing pressure were re-examined no nystagmus was seen and no one reported vertigo The middle ear volumes were calculated by the planimeter method and transferred from area (Am) to volume (Vm) The results of these calculations are given in Table V.

The results of the calculation of Ptm at the moment of passive forcing of the tubes are given in Table VI The table also includes the monaural recordings of the subjects

During ascents with active equilibration no nystagmus was seen and no vertigo was reported Descents with and without active equilibration caused neither vertigo nor nystagmus in any subject, nor was there any passive clearing of the ears when the subjects were exposed to descents All subjects could equilibrate static as well as dynamic over- and underpressures in a normal way and all belonged to tubal function groups Ib and Ic according to Elner et al (1971*b*)

It was also of interest to calculate the pressure gradient across the eardrum (Ptm) and the pressure difference between the ears at the moment when nystagmus started (Nystagmus pressure level–NPL) Table VII presents the NPL for each ear in the subjects with nystagmus during simulated ascents

Comment As seen from these results there is only a small variation in the pressure level at

Table VI *Pressure gradient across the eardrum (Ptm) at the moment of passite opening of the E chian tubes recorded at different sessions and with different numbers of examinations*

		Passive forcing cm H_2O					
		Ptm_{dx}			Ptm_{sin}		
Subject	Session no	*n*	Mean	Range	*n*	Mean	Range
III	1	3	21 7	21 4–22 0			
	2	7	25 8	24 6–27 8			
	3	2	29 3	28 0–30 6			
	4	1	29 0	29 0			
	5	3	33 3	31 7–34 6			
	6	10	28 2	24 9 34 6	10	16 3	15 6–18 6
IV	1	6	32 7	30 0–34 1			
	2	2	32 6	32 5–32 6			
	3	2	34 6	33 9 35 3			
	4	1	29 7	29 7			
	5	10	33 5	28 9–36 8	5	38 9	35 3–42 3
V	1	2	76.2	75 5–76 9			
	2	5	63 0	61 3–65 5	5	50 9	46 0–56 0
	3	8	59 6	51 0–65 6	8	44 1	37 3 56 0
VI	1	3	71 8	68 5–74 1			
	2	6	70 6	65 4–74 1	6	47 6	43 1 50 2
	3	8	67 2	63 8 70 9	8	44 1	39 8–49 9
VII	1	1	70 9	70 9			
	2	3	60 8	59 4–62 2	3	66 1	65 2–67 9
VIII	1	2	79 4	76 6–82 2			
	2	9	70 4	68 1–73 4	9	47 6	38 6–54 4

which nystagmus starts within each subject, recorded at different tests The magnitude of the asymmetry did not seem to influence the degree vestibular stimulation in subject II The great of the asymmetry in this subject is not ed by any variation of the forcing pressure the left hand side, but by the fact that the left ear was not cleared before the ascent (test 2, third examination) The subject had a relative underpressure in that ear at the beginning of the ascent

DISCUSSION

The present study shows that it is possible to elicit A V in a pressure chamber in certain subjects when exposed to simulated ascents with passive clearing of the ears The experimental situation is close to conditions that may be encountered in actual flying and diving, as most pilots and divers usually do not clear their ears actively during ascents Some authors are however of the opinion that even an ambient pressure increase (descent) might cause vertigo (Ar strong & Heim, 1937, Benson, 1965, DeWe & Saunders, 1960) This condition, howe could not be reproduced in the present study spite of a pressure increase of about 90 cm H in 25 sec, which in several subjects caused a re tive underpressure in the middle ears of ab the same magnitude It also appears from t present study that it was possible to elicit A in 5 out of 79 subjects all regarded as norma All of these 5 subjects had a high forcing pre sure on one side when compared with the me value of the whole material Repeated exam tions with intervals of several months reveal that the high forcing pressures could be repr duced and the individual variation of the forci pressures was low Asymmetry between the le and the right ear was also seen Asymmet between the ears with regard to the ease with whi pressure equilibration takes place has earli been recognized in interview studies of vert prone divers and flyers (Lundgren, 1965 Lu

able VII *Chamber pressure decrease* (ΔPch), *pressure gradient across the eardrum* (Ptm) *and the* .. *re difference between the ears at the moment the start of nystagmus was recorded* (*Nystagmus* ..*ssure level–NPL*)

go incidents and nystagmus intensity are also given Nystagmus was directed to the right in all examinations those indicated by *

bject	Session	Nystagmus pressure level – NPL (cm H_2O) ΔPch	Ptm_{dx}	Ptm_{sin}	$Ptm_{dx} - Ptm_{sin}$	Max velocity of eye-deviation in slow phase (°/sec)	Vertigo
I	2	69	64 4	Not recorded		3	+
		73 5	68 7	Not recorded		3	+
	3	66	61 3	16	45 3	4 5	+
		64 5	59 8	17 3	42.5	3	+
		66	61 3	15 5	45 8	5	+
		66	61 3	16 1	45 2	5	+
		69	64 1	16 9	47 2	3.5	+
II	2	66	59 3	Not recorded		2	+
		67 5	60 7	Not recorded		3.5	+
	3	57	51.2	32.3	18 9	1	+
		64 5	58 2	14 5	43 7	1	+
		60	54	22 7	31 3	0 5	+
	4	60	54	24	30	2.5	+
VI	2	66	61 1	12	49 1	2	+
VII	2	64 5	40 5	60 7	20 2	3 5 *	+
		70 5	40 5	66 4	−25 9	2 *	−
VIII	2	66	61	16 5	44 5	0 5	−
		72	66 5	21	45 5	0 5	−
		67 5	62.3	16 5	45 8	1 5	+

ren & Malm 1966) In the present study, however, it was difficult to estimate the importance of the degree of the asymmetry for eliciting vertigo As is seen from the results (subject II), oughly the same vestibular stimulation was reorded during two different tests though there was a great variation in asymmetry between the ars (examination 1 and 2 in session 3, cf Table VII) It is also seen from the tests with subject VII that despite a much smaller asymmetry, as ompared with that of the other subjects, inner ar stimulation was recorded

The mechanism for A V is, however, still un nown It has been suggested (Melvill Jones, 957 Benson, 1965), that the vertigo might be nduced by sudden movements of the stapes due o a sudden pressure change in the middle ear ollowing regulation or Valsalva's manoeuvre

It is difficult, however, to connect this brief stimulation with the duration of the vertigo, which by some subjects is reported to last for 15 minutes (Lundgren, 1965, Vorosmarti & Bradley, 1970) It appears from Fig 5, however, that the vestibular stimulation is not induced at the moment of pressure regulation as an effect of sudden movements of the stapes, but during the period of asymmetric middle ear pressure and not until the relative overpressure in the "notcleared" ear has reached a certain level This observation means that there might be another explanation of the way in which the pressure acts upon the inner ear than that previously suggested, or possibly that A V might be elicited in more than one way If the vestibular stimulation is caused by a cupula deviation it has to be by way of a fluid movement in the laby-

rinth Anatomically it is reasonable to expect that large displacements of the stapes footplate might cause a fluid flow in the inner ear However, when overpressure in the middle ear is considered as the cause of vertigo, it should be realized that the pressure acts equally on the two windows Then the labyrinthine fluid would not be set in motion unless there is a place for the fluid to escape Fluid displacement through the perilymphatic and the endolymphatic ducts might be considered However, the function of these structures is not known exactly Overpressure in the middle ear might also move the stapes footplate against the pressure gradient as the ossicles are mechanically linked to the eardrum and simultaneously push the membrane, covering the round window, so that it bulges into the inner ear, in this way setting the inner ear fluid in motion The function of the ossicular chain in such a case is however not exactly known, as the joints between the ossicles might "slip" when the movements of the eardrum are of large amplitude

As for the mechanism through which overpressure in the middle ear may induce vertigo and nystagmus, a circulatory insufficience in the inner ear caused by overpressure is also suggest ed Blood vessel connections between the middle r and the inner ear structures have been onstrated (e g Hansen, 1971), and as the pres level is recorded to be about 60 cm H_2O in re case of vestibular symptoms, there is a possibility of a hypoxaemia due to venous stasis and/or arterial insufficience

Another observation in the present study was that the subjects with a high forcing pressure on the right hand side got nystagmus to the right, and one subject (subject VII) with the highest pressure in the left ear got nystagmus to the left It should also be observed that the vestibularly induced eyeball deviation starts before the subject observes the stimulation and that nystagmus was recorded in some tests although the subjects did not observe vestibular stimulation

It is seen from the present study that certain subjects, although otherwise otologically normal, are more susceptible to experience of A V because of an inclination to a permanent h forcing pressure on one side A normal forc pressure might however be influenced and creased by different factors such as catarr infections, other inflammatory conditio oedema due to repeated barotrauma or ven stasis of different origin for example caused different positions of the body (Ingelstedt et 1967, Rundcrantz, 1969) It is seen from c reports that a great many subjects with expe ence of A V have also reported common co in connection with these symptoms (Mel Jones, 1957, Benson, 1965, Lundgren, 19 Vorosmarti & Bradley, 1969, Brown, 1971)

The method presented above might be use for improving flying and diving safety by eli nating subjects susceptible to vertigo caused middle ear mechanics as described in this pap It appears that it is not possible to make su a selection by using "ordinary" tubal functi tests only, but that the passive equilibrati capacity must be tested The capacity of passi forcing is not a tubal function in a physiologi sense and should not be confused with such function, since no active muscle work is involv in opening the tube There is no correlati between the active and passive equilibration ar as is seen in the present study the subjects w a high forcing pressure could all equilibrate a normal way When using the method presente above in routine examinations of otologicall healthy subjects, it seems sufficient to determ the ambient pressure change (ΔPch) needed force the Eustachian tubes open, as there w be only small differences between Ptm and ΔP as long as the middle ear volume (Vm) is not t small (<6 ml, see equation 1 and 2) This mea that the area of the airfilled middle ear cav should not be less than about 13 cm^2 It furt seems possible to prevent A V by freque equilibration during ascents also, since no ny tagmus was recorded in tests with vertigo-pro subjects at ascents with active equilibration

Statistical Comment

In the evaluation of the individual variation the passive forcing pressure (cf Table I) a m

ith two variance components was assumed σ_s^2 for variance "between sessions" and σ^2 for variance "between examinations within sessions". The variance components were estimated jointly from all cases. The estimates $\sigma_s^2 = 19.5$ and $\sigma^2 = 13.0$ were obtained. The corresponding standard ... are $\sigma_s = 4.4$ and $\sigma = 3.6$.

An analysis of variance for each case was performed, i.e. the sums of squares "between sessions" and "between examinations within sessions" were calculated. The estimates of σ_s^2 and σ^2 were based on the sum of the sums of squares 'between sessions" for all cases and the sum of the sums of squares "between examinations within sessions" for all cases (Snedecor & Cochran, 1973*b*).

ZUSAMMENFASSUNG

Bei 5 von 79 otologisch gesunden Versuchspersonen konnte durch simulierten Aufstieg und passiven Druckausgleich Schwindel hervorgerufen werden. Die experimentelle Situation glich sehr der Wirklichkeit, da die meisten Piloten und Taucher während des Aufstiegs aktiven Druckausgleich vornehmen, sondern einen passiven Ausgleich abwarten, der eintritt, wenn die des relativen Überdrucks im Mittelohr gross genug ist, um die Eustachische Tube zwangsmassig zu öffnen. Bei den Versuchspersonen, die Schwindel angaben, verglichen mit dem Mittelwert von 79 normalen Versuchspersonen einseitig ein erhohter Öffnungsdruck ... Tube festgestellt werden. Während der Dauer des Schwindels wurde regelmässig ein gleichseitiger Nystag... nus festgestellt. Diese vestibulare Stimulation wurde nicht im Augenblick des Druckausgleiches und somit durch die Wirkung schneller Stapesbewegungen ausgelost. Vielmehr erfolgte der Nystagmus während der gesamten Entspanne unsymmetrischen Mittelohrdruckes, aber erst nachdem der relative Überdruck im „nichtausgeglichenen" Ohr ein gewisses Mindestmass erreicht hatte.

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VESTIBULAR SYMPTOMS IN IDIOPATHIC FACIAL PALSY

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lbstract One week after the onset of the paralysis 75% espectively 92% of the patients with Bell s palsy presen ed with a spontaneous or/and positional nystagmus The pontaneous vestibular symptoms are discrete and usually iot subjectively realized by the patients No direct corre- ation between the degree of the vestibular disturbance nd the severity of the facial nerve palsy has been found n the first week after onset of the paralysis Only the ime evolution of the spontaneous vestibular signs (partic- larly of the positional nystagmus) showed whether the sion of the VII nerve was reversible or not The present tudy does not confirm therefore the hypothesis that the estibular signs present in Bell s palsy may be used for ie prognostic assessment of the lesion The quality and volution of the vestibular signs found in Bell s palsy o confirm, on the other hand, the surgical observation iat the most common site of lesion of the facial nerve i idiopathic palsy is the entrance of the Fallopian Canal nd not in its more distal course

'estibular symptoms occur quite frequently in resence of idiopathic facial palsy (Philipszoon, 962, Robert & Pfaltz, 1970) Their origin is iost probably related to the compression of ie superior branch of the vestibular nerve by ie edematous facial nerve fibres in the region f the meatal fundus (Fisch & Esslen, 1972, isch, 1973) The aim of the present study was determine the pattern and time evolution of ie vestibular disturbances observed in Bell's alsy and to analyse whether the vestibular mptoms correlate with the degree of severity the palsy

MATERIAL AND METHODS

ie spontaneous and positional nystagmus as ll as the caloric reaction of 25 patients (aver- e age 33 years) have been recorded by electro- nystagmography at regular intervals for 6 months after the onset of a unilateral idiopathic facial paralysis The caloric test was performed by irrigating the external ear canal with 20 cc of water of 27 at 47°C The following parameters of the caloric nystagmus were analysed dura tion, average maximal velocity of the slow component, frequency, and total maximal intensity In order to correlate the electronystagmographic findings with the degree of the palsy, the percentage of degenerated facial nerve fibres was determined by recording the summating potentials of the paralysed muscles of the face according to a method recently described by Esslen (1973)

RESULTS

The evolution of the spontaneous vestibular signs in 25 patients with idiopathic facial palsy are shown in Fig 1 One week after the onset of the paralysis 75% respectively 92% of the patients evidenced a spontaneous nystagmus or/ and respectively a positional nystagmus The average velocity of the slow component of the spontaneous nystagmus was, on average, 2–3 °/sec, its maximal velocity 5° per sec The number of patients with spontaneous vestibular signs decreasing rapidly in the first 4 weeks and slowly thereafter The percentage of patients with spontaneous nystagmus reached normal average values (16% of the cases) 4 months after onset of the palsy The positional nystagmus was still present in 40% of the patients at the end of the observation of 6 months The direction of the

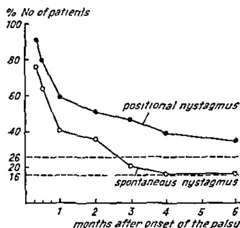


Fig 1 Time evolution of the spontaneous and positional nystagmus in 25 patients with idiopathic facial paralysis The broken lines represent the percentage of spontaneous (16 %) and positional (26 %) nystagmus found in a group of normal individuals with the same age as the investigated population

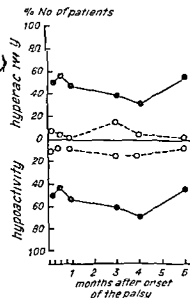
spontaneous vestibular signs did not change during the recovery from the palsy, pointing towards the healthy side in 2/3 and towards the affected side in 1/3 of the cases 20 % of the patients developed a direction changing positional nystagmus shortly before the disappearance of their spontaneous nystagmus

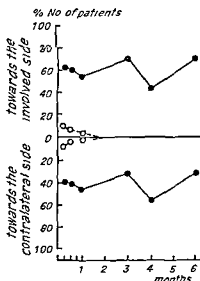
The analysis of the caloric response gave less clear results than that of the spontaneous vestibular signs The ear of the affected side wa hypoactive in one-half and hyperactive in t other half of the cases (Fig 2) Later on, t prevailing hyperactivity (second week) is fo lowed by a definite trend towards hypoactiv (third and fourth month) A similar pattern shown by the few cases (less than 20 %) hav a significantly reduced or increased calo response [1] The directional preponderance (Fi 2B) is mostly oriented towards the side of t palsy, particularly during the first 2 mont after onset of the palsy

Fig 3 shows the spontaneous vestibular sig in relation to the degree of the facial paralys Four groups of patients have been formed a cording to the results of the neuro-electr tests

(1) patients with axon-blockade only
(2) patients with less than 50 % degenerated facial nerve fibres

[1] According to the normal distribution of the calor nystagmus the unilateral response is significantly redu or increased if values of more, respectively less than 25 are obtained by the formula $(1+3)-(2+4)/1+2+3 \times 100$ %





●—● all cases   o-- o statistically significant cases

Fig 2 Time evolution of intensity of the caloric n tagmus (maximal velocity the slow phase) in 25 pa with idiopathic facial pa lysis Note that only a limited group of pati with Bell s palsy prese with significantly dis caloric response

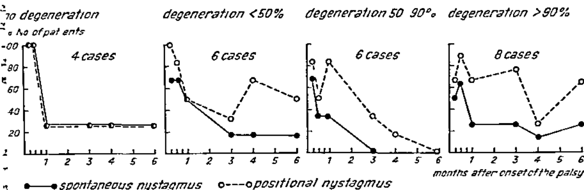


Fig 3 Relation between spontaneous and positional nystagmus and the degree of degeneration of the facial nerve in 24 patients with idiopathic palsy Note that there is no direct correlation between the degree of vestibular symptoms and the severity of the facial nerve degeneration during the first week after onset of the paralysis. The later time evolution of the spontaneous and particularly of the positional nystagmus are however delayed, particularly if more than 90% of the facial nerve fibres are degenerated

3) patients with 50 to 90% degenerated facial nerve fibres
4) patients with total degeneration of the facial nerve

The spontaneous vestibular signs disappeared more rapidly (4 weeks) in presence of an axon-blockade only than in patients with partial or total facial nerve degeneration

This observation indicated that in Bell s palsy the time evolution of the spontaneous vestibular signs reflects the degree of lesion of the nerve fibres

The correlation between the caloric nystagmus

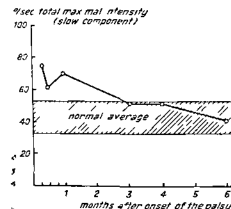


Fig 4 Evolution of the total maximal intensity of the caloric reaction of 25 patients with idiopathic facial paralysis Note the increased maximal intensity for as long as 3 months after onset of the paralysis

and the degree of the facial nerve lesion showed that the axon blockade is rather followed by the hypoactivity whereas the degeneration of the nerve fibres is followed by hyperactivity of the vestibular response on the affected side

The total maximal intensity of the caloric response (Fig 4) remained well above normal average values during the first three months of the palsy

DISCUSSION

75% respectively 92% of the investigated patients with idiopathic facial palsy presented with a spontaneous or/and positional nystagmus one week after onset of the paralysis (Fig 1) The direction of the spontaneous vestibular signs (towards the unaffected side) indicates that a rather severe lesion of the utriculo-ampullar nerve most probably due to compression from the edematous facial nerve (Fisch & Esslen 1972) takes place in 2/3 of the patients suffering from Bell s palsy The recovery from the vestibular disturbance is very rapid during the first 2 weeks after onset of the palsy and slows down thereafter The time-evolution of the spontaneous vestibular signs reflects therefore the acute phase of Bell's palsy, which is determinant for the destiny of the facial nerve fibres and rarely extends beyond the first 10–12 days after onset of the palsy (Esslen & Fisch, 1971)

The correlation between presence, respectively evolution of spontaneous vestibular signs and the degree of degeneration of the facial nerve fibres shows (Fig 3)

(1) that the spontaneous vestibular signs are more frequently observed in patients without than in patients with degeneration of the VII nerve, and

(2) that spontaneous and positional nystagmus disappeared more rapidly if the facial nerve fibres did not degenerate

According to these observations it is not the presence but *the time-evolution of the spontaneous vestibular signs* which correlates with the *severity of the facial palsy This is to be expected* since spontaneous and positional nystagmus can result from a reversible axon-blockade or from an irreversible degeneration of the afferent vestibular nerve fibres Only the time of recovery will permit a differentiation between these two different neural conditions It is also logical to suppose that the less important lesion producing a reversible blockade for impulse conduction in the facial nerve will also be followed by reversible changes in the fibres of the utriculo-ampullar division of the vestibular nerve

In view of the discrete character of the registered spontaneous vestibular signs it is not surprising that the caloric reaction of patients with *Bell's palsy is only insignificantly disturbed* This may also result from the fact that the intrameatal compression from the edematous facial nerve involves particularly the utricular nerve, which is closer to it and to a lesser extent the lateral situated superior and horizontal ampullar fibres

In conclusion, there is no direct correlation between the degree of the vestibular disturbance and the severity of the facial nerve palsy during the first week after onset of the paralysis Only the later time-evolution of the spontaneous vestibular signs (particularly of the positional nystagmus) shows whether the lesion of the VII nerve was reversible or not The present study does not confirm, therefore, the hypothesis that the vestibular signs present in Bell s palsy may be used for the prognostic assessment of the lesion The quality and evolution of the ve lar signs found in Bell s palsy do confir the other hand, the surgical observation th most common site of lesion of the facial in idiopathic palsy is the entrance of the pian Canal and not its more distal course

ACKNOWLEDGEMENT

We acknowledge the technical assistance of Mrs Regeler

ZUSAMMENFASSUNG

Ein Spontan- oder Lagenystagmus wurde bei 75 % r tive 92 % von 25 Patienten mit einer einseitigen

Storung und Schwere der Parese konnte in der Woche nach Einsetzen der Läsion nicht nachge werden Der weitere Verlauf der vestibularen Stor (vor allem der Lagenystagmus) zeigte dagegen Lasion des Gesichtsnerven reversibel war oder nich vorliegende Untersuchung zeigt dass aus der vestib Symptomatik kleine Schlusse in bezug auf die Pro einer Bell schen Parese gezogen werden konnen D und der Verlauf der vestibulären Zeichen bei einer schen Parese bestatigen, dass der Ort der Läsio haufigsten am Anfang des Fallopischen Kanals und weiter distal liegen muss

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POSITIONAL AND POSITIONING NYSTAGMUS AS A RESULT OF UTRICULOCUPULAR INTEGRATION

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Abstract A short review of the positional nystagmus problem is given The different planes of position for obtaining positional nystagmus are described and its different types discussed The results of experiments during recent years on animals and on man are presented and clinical conclusions are drawn from these results Positional and positioning nystagmus are defined, and the most suitable positions for positional nystagmus are mentioned Normal and pathological results which are to be expected from the labyrinth during these positions are presented A method of studying positioning horizontal nystagmus as a result of integrated utriculo-cupular activity is described and the results discussed

Positional nystagmus is generally defined as a spontaneous nystagmus which is not constantly present, but appears only in certain positions (Barany, 1913, Voss, 1921, Borries, 1923, Gerlings, 1948, Nylen, 1950) To this definition Aubry et al (1954) want to add one more group with spontaneous nystagmus in normal positions, which changes appearance or intensity in a certain position of the body

As early as 1913 this phenomenon was described by Barany, who in 1924 was able to show that these reactions were released by position and not by movement The problem has subsequently been elucidated by hundreds of authors, who stated that positional nystagmus is observed in diseases of many different kinds both of the peripheral labyrinth and of the central nervous system (See Frenzel, 1961*a*)

Planes of position

The method used for investigating positional nystagmus varies from author to author, but principally the investigation is made in two different planes (Nylen, 1950, Aubry et al, 1954)

1 The sagittal plane in a sitting or supine position, or with the head hanging out over the upper edge of the investigation table

2 The frontal plane, in a sitting position with the head tilted over one shoulder, or in a right or a left lateral supine position

Nylen recommends that the patient's head and body should move together to exclude the so-called neck reflexes and that change of position should take place very slowly Usually, the position should be changed 90° in 5 sec However, for scientific purposes he recommends a slow constant speed of 1–2° per sec without any acceleration Dix & Hallpike (1952), Cawthorne (1954) and Cawthorne & Hallpike (1957) recommend that from a sitting position the patient should be rapidly be brought, within 3 sec, to a supine position with the head turned to either side The head is moved independently of the body Cawthorne considers that this has no importance for the results

Types of positional nystagmus

By using the above mentioned methods of investigation positional nystagmus has been studied in a great many diseases both of the peripheral labyrinth and of the central nervous system It has been shown that positional nystagmus can be classified according to different types Nylen has been one of the pioneers in this field Between 1924 and 1950 he published

several papers on positional nystagmus He proposed the following classification

Type 1 Direction-changing, positional nystagmus, which is characterized by a nystagmus changing direction in different head positions Consequently, in one position nystagmus can be horizontal, and in the opposite position vertical or rotatory

Type 2 Direction fixed, positional nystagmus, where nystagmus is invariably in the same direction, when the subject is in different positions If nystagmus is observed in all head positions, it is definitely altered in intensity in a certain position

Type 3 Irregular, positional nystagmus, characterized by variations in its behaviour

Other authors have made similar classifications, which, however, except for minor variations, coincide with the above mentioned classification (Ruttin, 1936, Seiferth, 1937, Frenzel, 1938, Lindsay, 1951, Aubry et al, 1954)

Most scientists seem to agree that positional nystagmus Type 2 usually occurs in peripheral labyrinthine diseases (Blomqvist, 1948, Gerlings, 1948, Nylen, 1950, Cawthorne, 1954, Aubry et al, 1954 Lindsay, 1951), even though this type also occurs in central diseases (Fromm 1934, Nylen, 1939, 1950) Type 1 and Type 3 are mostly observed in central diseases of different kinds

However, the different authors have not only described the type of positional nystagmus they found but also its appearance and duration Barany described a case with horizontal nystagmus to the left in a right lateral position In 1921 he published a report on another case with rotatory nystagmus with the upper pole of the eyes beating to the right in a right lateral position Voss (1921) has observed horizontal nystagmus to the left, when the patient leaned his head over his right shoulder Ruttin (1936) and Vogel (1950) described several labyrinthine injuries, with rotatory, positional nystagmus in the same direction as the patient is tilted The duration was only a few seconds Nylen (1950) observed a rotatory nystagmus in one lateral position and a horizontal nystagmus in the other lateral position The nystagmus was usually considered to be tonic, i e, inexhaustible but sometimes he found that the duration of the nystagmus was only a few seconds Dix & Hallpike (1952) and Cawthorne & Hallpike (1957) described rotatory or horizontal rotatory nystagmus beating towards the undermost ear with a duration not exceeding 10 sec Michels (1952) made the same observations and found that the duration was about 15 sec Aubry et al (1954) found that positional nystagmus continued for as long as the patient held a certain position, but sometimes the durations were only 30–40 sec This problem has been specially studied by Stenger (1955), who distinguishes between "Lagenystagmus" and "Lagerungsnystagmus", i e, nystagmus in a certain position and nystagmus immediately after positioning the patient in a certain position He observed rotatory or horizontal-rotatory nystagmus and considers that, as a rule, positional nystagmus is inexhaustible, whereas positioning nystagmus has a duration of only a few seconds

It is evident that all these authors have observed either a horizontal or a rotatory nystagmus, or a combination of both In those cases where the duration is mentioned, this is generally so short, that one must question whether the nystagmus was not due to the interference of angular acceleration The same opinion has been expressed by Frenzel (1961*b*) and Jongkees (1961)

The peripherally induced positional nystagmus

Even if the cause of positional nystagmus is to be found peripherally as well as centrally it is established that a functioning labyrinth at least on one side, is a prerequisite for elicitation of spontaneous as well as positional nystagmus It is known that bilateral elimination of labyrinthine function extinguishes both these reactions (Spiegel & Scala, 1942, Dow, 1938 Aschan & Fernandez, 1960) Rotatory eye movements are absent in bilaterally labyrinthectomized patients (Miller & Graybiel 1963) Equally, alcohol nystagmus does not appear in patients without labyrinthine function even though a

spite of this, they are capable of becoming drunk (Aschan, 1961). Since the vestibular apparatus is a differential organ (Fluur & Mellström, 1971) i.e. it is the difference in input from the right and left labyrinth, which causes the ocular reactions, one has good reasons to assume that positional nystagmus of peripheral as well as central origin depends on a disturbance of the balance between the activity of the right and left vestibulo-ocular reflex arcs, and that the trigger mechanism for spontaneous as well as positional nystagmus is localized to the peripheral labyrinth. Therefore the present author has been interested in positional and positioning nystagmus, induced by interaction of input from the semicircular canals and otolith organs by tilting and which, so to say, give reactions according to the rules for a peripherally induced alteration of activity, whether this is normal or pathological.

As early as 1921 Bárány thought that positional nystagmus was a disorder of the otolith apparatus. The same opinion was held by Voss (1921), and later also by Chilow (1927). Hasegawa (1939), de Kleyn & Versteegh (1930) and Dix & Hallpike (1952). Dix & Hallpike (1952) and Cawthorne & Hallpike (1957) have described histological findings, which favor the belief that the damage is localized in the utricle. Later, Schuknecht (1969) demonstrated histologically that positional nystagmus can also arise by substances of high specific gravity, possibly otoconia, acting upon the cupula of the posterior semicircular canal.

Earlier the utricular effect on oculomotion was not known in detail, and furthermore, it was not known if the otolith organs really are capable of inducing nystagmus. However, investigations made during recent years, have thrown new light on this problem. Fluur & Mellström (1970) have shown that electrical stimulation of different areas on the utricular surface cause distinct eye movements. Subsequently Fluur & Siegborn (1973) in cat and Fluur (1973) in man, have demonstrated that these different areas co-operate with the semicircular canals in an intimate and lawful way in their influence on the oculomotor system. The results can be summed up as follows. After a unilateral labyrinthectomy, horizontal nystagmus is facilitated if the patient is tilted around the longitudinal axis towards the diseased ear, and is inhibited if he is tilted towards the sound ear. After selective sectioning of the two anterior ampullar nerves in cats, the vertical nystagmus upwards is facilitated by tilting head upwards around the bitemporal axis. On the other hand, selective sectioning of the two posterior ampullar nerves induces a vertical nystagmus downwards, which is facilitated by tilting the animal's head downwards. Tilting in the opposite direction inhibits nystagmus in both these cases. Unilateral sectioning of the two vertical ampullar nerves gives a rotatory nystagmus with the upper pole of the eyes beating towards the sound ear. Tilting the animal around its longitudinal axis towards the sound ear facilitates this nystagmus whereas tilting in the opposite direction causes an inhibition. Finally, unilateral selective sectioning of all the three ampullar nerves induces a horizontal rotatory nystagmus. In one lateral position the horizontal component is prevalent, in the other the rotatory.

CONCLUSIONS

From all these results the following conclusions can be drawn. A unilateral labyrinthine disease can, during tilting around the longitudinal axis of a patient in supine position, induce a horizontal nystagmus, if the patient is tilted towards the affected ear, and a rotatory nystagmus (with the upper pole of the eyes beating towards the sound ear) if the patient is tilted towards the sound ear. These findings coincide completely with clinical results obtained by Barany, Ruttin, Voss and Nylen and are classified as Type 1.

A very important finding of the animal experiments is that a horizontal, positional nystagmus released from the labyrinth invariably beats in the opposite direction to that in which the animal is tilted. These findings also coincide with Nylen's Type 2.

Concerning Type 3 it can immediately be stated that the experimental investigations have

shown a clear relation between position and distinct eye movements Therefore, if a patient during one and the same investigation manifests an irregular positional nystagmus, this indicates a nonlabyrinthine disease On this most authors also agree

Here, it may be appropriate to point out that it is not possible to 'reverse" the problem i e, to draw, from a certain finding, the conclusion that the damage is localized in this or that peripheral labyrinth, since it must always be borne in mind that pathological processes may be present at any place in the reflex arc and still display the same system This is why Types 1 and 2 are found even in diseases of the central nervous system

Definitions

The above-mentioned experimental investigations have now enabled us to indicate an appropriate method for investigating positional and positioning nystagmus, and also to state the results which can be expected from the peripheral labyrinth However, it is imperative to first clearly define what is meant by these expressions

1 Positional nystagmus (Lagenystagmus) is a static reaction, appearing in certain positions without preceding angular acceleration

2 Positioning nystagmus (Lagerungsnystag-) is a dynamic reaction, due to combined lar and linear acceleration, or to lymphonetic movements in the peri endolymphatic system, stimulating the peripheral sense organs

Methods of investigation

The fact that the otolith organs and the semicircular canals are intimately integrated in their activities and their influence on oculomotion, makes certain demands on the method of investigation

A In order to get answers which are as clean as possible, preferably concerning only one of the rotation axes of the eye, the stimuli must be applied in distinct planes in relation to the sensory cells, which are to be stimulated

B A clean stimulation of the otolith organs presupposes the absence of stimulation of semcircular canals The angular acceler which brings the patient into the desired posi must always be subthreshold This can onl done by means of electrically-driven rot tables, making very slow accelerations pos Such a table, specially made for the purpo manufactured by MSA (Stille-Werner)

C Concerning the test for positioning ny mus the intensity of the stimulus must be known and reproducible

The most suitable positions for positional tagmus are

1 Lateral position, which can give rise to l zontal or rotatory nystagmus

2 Supine or prone position, which only vertical nystagmus To place the patient his head hanging backwards does not in an increased stimulation of the otolith or and can, from this point of view, be discar Possibly, this position can affect the muscle the neck or the vertebral arteries or have a l phokinetic effect via the cochlear aqueduct thus supply information of a primarily vestibular character

Normal and pathological results to be expe from the labyrinth

If the saccule, which causes vertical eye m ments (Fluur & Mellstrom, 1970*b*), is not ta into consideration, our present knowled the utricular function entitles us to count on following results during normal and patholog conditions The *g* force must be regarded vector, which, in different positions, char direction over the utricular surface (Fig 1)

POSITIONAL NYSTAGMUS

Supine position

Normal patients In this position there is an creased activity from the anterior lateral posterior medial areas of both utriculi, w causes a depression of the eyes Simultaneo the posterior lateral and the anterior me areas, which enable elevation, are inhibited midlateral areas, which account for horizo

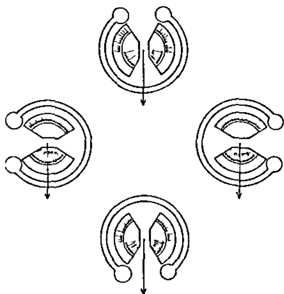


Fig 1 Schematic diagram of the horizontal semicircular canals and the utricles in different positions as seen from above The small arrows on the utricular surfaces indicate areas of increased activity in a certain position The large arrows indicate the direction of the *g* force

eye movements, do not have any effect on the eyes, since there is no difference in activity between the two sides

Acute unilateral damage of the labyrinth Only horizontal nystagmus towards the sound ear is obtained Vertical nystagmus is not to be expected, because both elevation and depression are represented on only one utricle On the other hand, unilateral damage can give rise to a rotatory component of nystagmus However, horizontal nystagmus preponderates over vertical and rotatory nystagmus

Compensated unilateral damage of the labyrinth No nystagmus or nystagmus only with open eyes in darkness or behind closed eyes and with electronystagmographic recording

Right lateral position

Normal patients Here there is increased activity in the lateral part of the left utricle, and in the medial part of the right (Fig 1) This causes a horizontal deviation of the eyes to the right and, simultaneously, a clockwise rotation

Acute unilateral damage of the labyrinth If the damage is on the right side, there is an increase in the frequency of the horizontal, left-beating nystagmus A leftsided damage causes a counterclockwise rotatory nystagmus (Nylén, 1950, Fluur & Siegborn, 1973)

Compensated unilateral damage of the labyrinth. Here positional nystagmus is horizontal if the damage is right-sided and counterclockwise rotatory if the damage is left sided

Prone position

Normal patients Increased input is obtained from the anterior medial and posterior lateral areas of both utriculi (Fig 1), and the patient's eyes are elevated Simultaneously, the anterior lateral and posterior medial areas, which are responsible for depression, are inhibited The "horizontal" components are not influenced for the same reasons as in the supine position

Acute unilateral damage of the labyrinth This causes a spontaneous horizontal nystagmus towards the sound ear, sometimes with a rotatory component with the upper pole of the eyes beating in the same direction

Compensated unilateral damage of the labyrinth No nystagmus or nystagmus only with open eyes in darkness or behind closed eyes and with electronystagmographic recording

Left lateral position

Normal patients There is an increased input from the lateral part of the right utricle and from the medial part of the left (Fig 1), which causes a horizontal eye deviation to the left and a counterclockwise rotation

Acute unilateral damage of the labyrinth This increases the right-beating nystagmus, if the left labyrinth is damaged, or a clockwise, rotatory nystagmus if the damage is on the right side (Fluur & Siegborn, 1973)

Compensated unilateral damage of the labyrinth This induces positional nystagmus, horizontal when the left side is damaged, and clockwise rotatory in right-sided diseases

POSITIONING NYSTAGMUS

The term "positioning nystagmus" is commonly used for a nystagmus induced by a rapid alter-

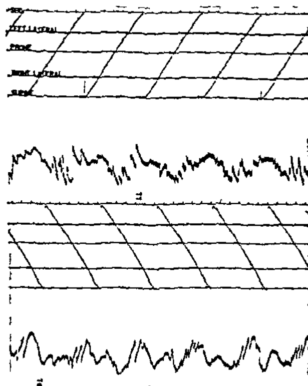


Fig 2 Electro-nystagmographic recordings from a normal person during constant rotation at a speed of 60°/sec around the longitudinal axis The upper half of the curves indicates the rotation of the table, the lower half indicates *the horizontal nystagmus The upper curves show* nystagmus towards the right during clockwise rotation, inhibited in the right lateral position, and facilitated in the left lateral position The lower curves show nystagmus towards the left during counterclockwise rotation, inhibited in the left lateral position and facilitated in the right lateral position

tion of position in the sagittal plane according to Dix & Hallpike (1952), Cawthorne (1954) and Stenger (1955) The positioning procedure used by Stenger with a movement only in the sagittal plane involves a stimulation of the utricles and the two anterior or posterior semicircular canals and ought to give vertical nystagmus The rotatory nystagmus obtained by him must therefore have another cause According to the author this movement can give rise to lymphokinetic movements in the peri endolymph via the cochlear aqueduct, most often producing a rotatory nystagmus of short duration and without reproducibility

On the other hand, the procedure used by Dix & Hallpike with a movement in the sagittal plane, combined with a turning of the head to either side, could very well induce such a rotatory nystagmus by an ampullofugal endolymph movement in the two vertical canals of the labyrinth and on ampullopetal movement in the same canals of the contralateral labyrinth Therefore it is an excellent test of the utriculo-semicircular integrations in benign paroxysmal positional nystagmus, by the authors supposed to have its cause in the utricle

Sticking to the definition of positioning nystagmus as a dynamic reaction, a slow alteration of position of a supine patient with a rotation around his longitudinal axis introduces a new *kind of positioning horizontal nystagmus, hitherto* not used as a clinical test of the interaction between the semicircular canals and the otolith organs

We know that the otolith organs *per se*, without the presence of semicircular canals are capable of inducing nystagmus (Fluur & Siegborn, 1973*d*) The otolith organs are dependent on functioning semicircular canals, the input of which is modulated by changing the utricular activity Therefore, a combined stimulation of the semicircular canals and the otolith organs is a good method for studying the cooperation

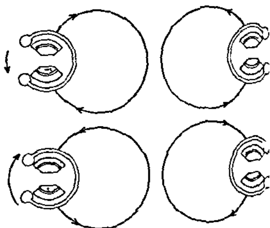
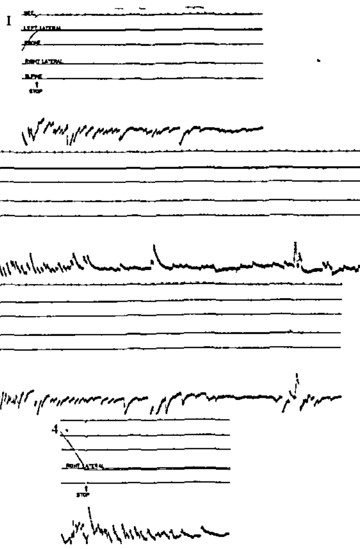


Fig 3 Schematic diagram of the horizontal canals and the utricles, as seen from above, when rotation around the longitudinal axis of the subjects is suddenly stopped The circular arrows indicate the direction of rotation The smaller arrows at the side indicate the intensity and direction of the horizontal nystagmus after this rotation The small arrows on the utricular surface indicate areas of increased activity in a certain position



ig 4 Electro-nystagmographic recording of postrotatory orizontal nystagmus from a normal person when otation at a speed of 60 /sec is suddenly stopped, in ft (curves 1 and 2) or right (curves 3 and 4) lateral osition after clockwise (curves 1 and 3) or counter-lockwise (curves 2 and 4) rotation The upper half of one curve shows the position of the table, the lower half the direction and intensity of nystagmus In the positions where the utricles and the semicircular canals are functioning synergistically the duration of postrotatory nystagmus is much longer—about twice—than in the positions where they are functioning antagonistically

etween the horizontal semicircular canals and ie utricles in their influence on the oculomotion With an electrically-driven rotation table the atient can, in supine position, be rotated around is longitudinal axis to the right or the left with r reproducible acceleration Through the simul-aneous alteration of the direction of the g vector n the utricular surface the input from the utricle modulates the nystagmus induced from the horizontal canals In the supine position there is indeed an influence on those sensory cells which cause vertical eye movements, but during increased tilting, the effect of the g-force increases with the sine of the angle on those cells which give rise to horizontal or rotatory eye movements The output from the sensory cells increases namely with the cosine of the angle between the direction of applied displacement of

the sensory cells and the direction of maximum sensitivity (Flock, 1965) In supine position, these cells are in a "spontaneous" position, whereas in a lateral position they are in "optimal"-position

If the patient is accelerated to the right, nystagmus beating to the right occurs In the right, lateral position an antagonism develops between the input from the left utricle and the right horizontal canal which somewhat inhibits the nystagmus In the left lateral position, on the other hand, the input from the right utricle and right horizontal canal functions synergistically, which increases the intensity of the right-beating nystagmus (Fig 2) The table can also be stopped after moving at a constant speed to the right or left (Fig 3) Then, by stopping in a right lateral position after rotation to the right postrotatory nystagmus to the left is developed in the patient, which is facilitated by the simultaneously increased input from the left utricle If the table is stopped in the left lateral position after rotation to the right, the postrotatory nystagmus to the left is inhibited by the increased input from the right utricle By comparing the results from the right and the left lateral position after rotation in both directions, information is obtained about the integration of the activity between the utricles and the horizontal canals (Fig 4)

If there is any value in investigating the integration between the utricles and the vertical canals, this can be done by rotating the patient in the sagittal plane from a sitting position at a known acceleration forwards or backwards, to a horizontal position In this case the utricles and the vertical canals are operating synergistically, producing a clean simultaneous angular and linear stimulation, which under normal conditions results in vertical nystagmus Since the activity of the cells which cause horizontal eye movements is on the same level bilaterally, horizontal nystagmus is never obtained by this stimulation

The recording of the eye movements is a question of utmost importance, since it is impossible to record rotatory eye movements electronystagmographically Visual inspection of the eyes behind Frenzels glasses in darkness is theref recommended, together with nystagmogra recording of horizontal and vertical nystagm However, a disadvantage in recording ver nystagmus is that closing the eyes can ind vertical nystagmus by itself without vestibu stimulation, which makes it more difficult interpret the nystagmus from the labyri (Fluur & Mendel, 1963) Therefore such reco ing ought to be done with open eyes in the da

ZUSAMMENFASSUNG

Es wird eine kurze Übersicht uber das Problem Lagenystagmus gegeben Die verschiedenen Positio zur Hervorbringung eines Lagenystagmus werden schrieben und die verschiedenen Typen diskutiert I Resultate von Versuchen an Tieren und Menschen w rend der letzten Jahre werden vorgelegt und die klinisc Schlusse gezogen Lage und Lagerungsnystagmus wer definiert und die geeignetsten Positionen fur Lagenys mus genannt Normale und pathologische Ergebni die bei diesen Positionen vom Labyrinth zu erwar sind, werden dargelegt Eine Methode die den horiz talen Lagerungsnystagmus als Ergebnis einer integrier utriculocupularen Aktivitat studiert wird beschrieb und die Ergebnisse werden diskutiert

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HISTOCHEMICAL LOCALIZATION OF ACETYLCHOLINESTERASE ACTIVIT IN THE COCHLEAR AND VESTIBULAR GANGLION CELLS

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Abstract The localization of AChE activity was investigated in the cochlear and vestibular ganglion cells of the chinchilla inner ear with the electron microscope by Karnovsky s method The reaction product was localized within the rough surfaced cisterns of the E. R including the perinuclear cistern and in some cisterns of the Golgi complex In a few myelinated nerve fibres and in most of the unmyelinated ones the reaction product was present along the axolemma The reaction product was absent in the satellite and Schwann cells The controls indicated that the reaction was due to AChE activity

After Churchill & Schuknecht (1959) and Schuknecht et al (1959), numerous other investigators confirmed, both at light and electron microscopical levels, that in the organ of Corti and in tibular sensory areas, acetylcholinesterase ChE) activity is strictly associated with the fferent nerve fibres and endings Under the same experimental conditions, the afferent nerve endings did not show any AChE activity (reviewed by Iurato et al, 1971*a*)

In the cochlear and vestibular ganglion cells, which give origin to the afferent nerve endings resulted negative at the periphery, some authors found evidence of AChE activity, but contradictory results were obtained by others In the cochlear ganglion cells Vinnikov & Titova (1964) Firbas & Welleschik (1970) Nakai (1972) and Firbas et al (1973) found a positive rea Schuknecht et al (1959) obtained a ne result, but they could not conclude that structures were free of AChE activity fo solutions probably did not penetrate to ganglion Rossi (1961) found a positive rea in the cells of the cochlear ganglion in the g pig, only in the early foetal development et al (1967*a*) demonstrated a positive ACh action in the saccular and cochlear ganglion of the human inner ear, but not of the mo cat, guinea pig (1967*a*) and squirrel mo (1967*b*)

On the other hand, in other sensory ga neurons (spinal and trigeminal) of adult young animals, AChE activity was demonst by using the light microscopic histoche methods, electron microscopic histoche methods and microchemical techniques viewed by Pannese et al, 1971, 1974)

The present research was undertaken to vestigate whether the neuronal bodies of coc and vestibular ganglion cells show in the c chilla AChE activity with a method (Kar sky's) which already gave clear-cut results in organ of Corti and in vestibular sensory of the same zoological species (Iurato et 1970, 1971*a*, 1971*b*)

In case of positive result it was of som terest to establish the precise sites of A activity in these neuronal bodies

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Additional objectives of the research were to ieck the behaviour in respect of AChE activity the two cell populations described in the ɔchlear (Kellerhals 1967) and vestibular (Bal ntyne & Engstrom 1969) ganglia and to con ol the existence of connections between the rve cells and of synapses in the same ganglia ı fact Adamo & Daigneault (1972) found finger ke intercellular projections connecting the eurons in the cochlear ganglion of the cat and hrenbrand & Wittemann (1970) described syn ptic nerve endings in contact with the vestibu r ganglion neurons in the mouse structures ot mentioned in the previous extensive stud es y Kellerhals (1967) and Ballantyne & Eng rom (1969)

MATERIAL AND METHODS

ive adult chinchillas were anesthetized with an ıdoperitoneal injection of sodium pentobarbital 35 mg/kg) Artificial respiration was maintained sing a Braun Melsungen apparatus The ani ıals were first endovascular perfused with 5 ml łaemaccel (pH = 7 2) then with a fixative con ining formaldehyde 2 % and glutaraldehyde 25 % in buffered 0 1 M sodium cacodylate ɔH 7 3) with 10 mg % $CaCl_2$ Immediately terwards the temporal bones were quickly re- oved The bony cochlea was widely opened ıd the stapes taken away to allow a good netration of the fixative The specimens were ft 2 hours in the same fixative used for the rfusion After the fixation was completed the ɔecimens were placed in the same previously ed buffer containing 0 22 M sucrose for 2 ɔurs and the dissection of the cochlear and st bular ganglia was performed

orphology

ɔme specimens containing cochlear ganglion lls and others with the vestibular ganglion re post fixed for 2 hours with 1 % osmium troxide buffered accord ng to Millonig (1961)

istochemistry

rom other specimens 75 μm thick sections were epared using a Sorvall TC2 tissue chopper These sections were rinsed in 0 44 M sucrose for 20 minutes then transferred into the in cubation medium (substrate acetylthiocholine (AThCh) iodide Hoffmann La Roche & Co Ltd Basel or Fluka AG Bucks St Gallen) prepared according to Karnovsky (1964) The incubation time was 30 minutes As a control for the histochemical reaction a number of thick sections was preincubated for 1 hour in a 2×10^{-5} M acqueous solution of BW 284 C 51 ([1 5 *bis* (4- allyl dimethylammoniumphenyl) pentan 3-one dibromide] Wellcome Research Laboratories Beckenham Kent England) These sections were then incubated for 30 minutes in a complete medium to which the inhibitor was added at the same concentration used for the pre incubation

After incubation all thick sections were rinsed in 3 changes of 0 44 M sucrose for 10 minutes They were post fixed for 30 minutes in 1 % os mium tetroxide buffered according to Millonig (1961) and then dehydrated in alcohol All these procedures were performed at 0–4 C Epon 812 was used as embedding medium Thin sections were cut with an LKB 4800 III ultramicrotome The thin sections were placed on Formvar car bon membranes (Dowell 1964) or on Formvar grids and observed with Siemens Elmiskop 101 and 1A and Zeiss EM 9A electron microscopes

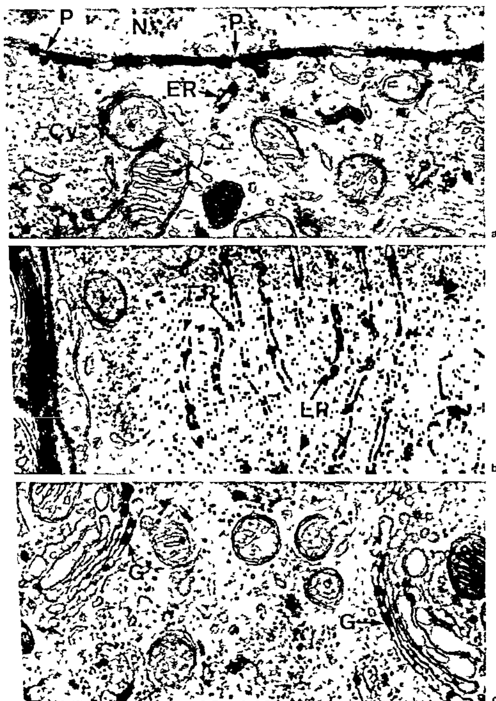
RESULTS

Locali ation of reaction product

Since the localization was similar in the cochlear and vest bular ganglion cells to avoid repe ion a unique description is given In the neuronal body most of the reaction product (Cu ferro- cyanide) was localized within the rough surfaced cisterns of the endoplasmic reticulum (E R) in cluding the perinuclear cistern (Figs 1 2*a* 2*b*) Reaction product associated with dense bodies was not observed Golgi complexes showing re action product could be observed (Fig 2*c*) to- gether with Golgi complexes devoid of reaction product in the same section of a neuronal body The reaction product was never observed along the perikaryal plasma membrane and the plasma membrane of the initial portions of the dendrite



Fig 1 Vestibular ganglion cell from a chinchilla The nucleus (*N*) is located centrally and contains one nucleolus (*Nu*) The surrounding cytoplasm contains rough surfaced cisterns of the endoplasmic reticulum and large numbers of organelles The cell is surrounded by a multilayered myelin sheath, consisting of both compact (M and loose myelin (*MyL*) *S* = satellite cell *C* = co space *UF* = unmyelinated nerve fibres The product is localized within the rough surfaced of the endoplasmic reticulum × 10 800



g 2 (*a*) Detail of a chinchilla vestibular ganglion cell s owing the nucleus (*N*) and a small part of cytoplasm)) *P* pore in the nuclear envelope The reaction prod- t is localized within the perinuclear cistern and in a tern of the rough endoplasmic reticulum (*ER*) × 52000) Detail of a chinchilla cochlear ganglion cell, sur unded by the myelin sheath (*M*) *C*) The granules of the reaction product appear localized within the rough-surfaced cisterns of the endoplasmic reticulum, arranged in parallel arrays (*ER*) × 44 000 (*c*) Detail of a chinchilla vestibular ganglion cell showing two Golgi complexes (*G*) The reaction product appears within some of the Golgi cisterns × 50 000

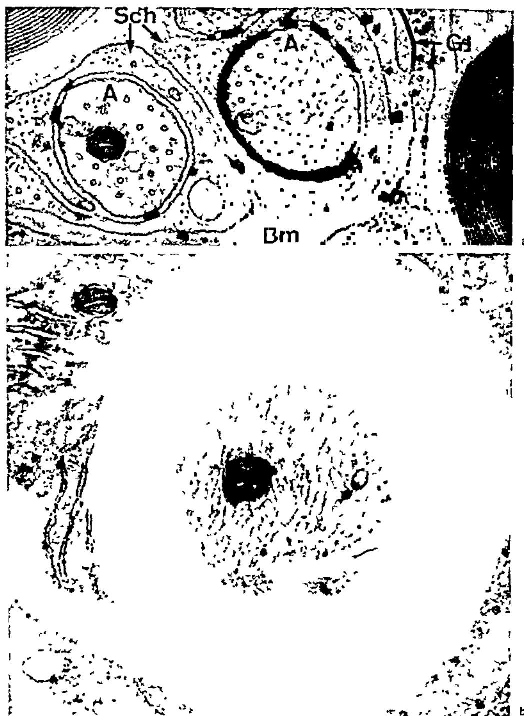


Fig 3 (*a*) Detail of the cochlear ganglion from a chinchilla showing two unmyelinated axons (*A*), containing micro tubules and filaments *My* myelin, *Sch* – Schwann cell, *GJ* = gap junction, *Bm* = basement membrane The reaction product is localized along the axok × 58 000 (*b*) Myelinated fibre in the cochlear g' from a chinchilla The reaction product is localized the axolemma × 62 000

d axon [1] In a few myelinated nerve fibres a amount of reaction product was present along the axolemma (Fig 3*b*) Most of the un el ' nerve fibres showed a large amount f the reaction product on their outer surface Fig 3*a*) The satellite and Schwann cells did not ontain any reaction product In agreement with et al (1974), who used the same tech nique to study the spinal ganglia of adult fowls, he above described pattern of localization of he reaction product could only be observed in he more superficial layers of the thick sections obtained with the tissue chopper, whereas in the nnermost layers of the thick sections neuronal bodies devoid of reaction product could be found n the innermost layers of the thick sections the eaction product was present in the cleft between he neuron plasma membrane and the related atellite cell sheath as well as in the cleft between he satellite cells

Controls

n any of the above mentioned sites no reaction vas obtained in the presence of 2×10^{-4} M BW 84 C 51 As BW 284 C 51 is an AChE selective nhibitor (Austin & Berry, 1953, Bayliss & Tod ick, 1956) further controls were not carried out

DISCUSSION

n both the cochlear and vestibular ganglion the eaction was positive in the following sites ough surfaced cisterns of the E R, including the erinuclear cistern and part of the Golgi com lex of the ganglion cells axolemma of a few nyelinated and of most of the unmyelinated erve fibres Since the reaction was positive by sing AThCh as a substrate without inhibitors nd negative by adding BW 284 C 51 as an in ibitor it can be concluded that it was due to AChE activity

In the present research the reaction was performed on 75–100 μm thick sections to allow the solutions to penetrate the ganglion and the myelin sheath of the ganglion cells In our material neuronal bodies which did not present any reaction product were observed only in the innermost layer of the sections On the basis of the results obtained, it is possible to conclude that AChE activity is present in the neuronal bodies of the cochlear and vestibular ganglion cells of adult chinchillas like in the sensory ganglia neurons of most adult animals (reviewed by Pannese et al, 1971, 1974) Therefore the negative results obtained in other species by Schuknecht et al (1959) Rossi (1961) and Ishii et al (1967*a* 1967*b*) could be possibly explained with the difficulties the solutions have to penetrate the ganglion and the myelin sheath of the ganglion cells

The sites where AChE activity was demonstrated in the present research are the same found by numerous authors in the sensory ganglia and in other regions of the nervous system (reviewed by Pannese et al, 1974)

Both the myelinated and the unmyelinated ganglion cells in the vestibular ganglion of the chinchilla showed the reaction product Therefore no difference in respect of AChE activity was evidenced in the two cell populations of the vestibular ganglion In the cochlear ganglion of the chinchilla we did not find a single unmyelinated cell, but this is probably due to the limited number of sections observed

The morphological controls fail to demonstrate any type of contact between the cochlear ganglion cells and of synapses around the vestibular ganglion cells Our results are therefore in agreement with those of Kellerhals (1967) and Ballantyne & Engstrom (1969) and not with those of Adamo & Daigneault (1972) and Ehrenbrand & Wittemann (1970) Synapses were found in the vestibular specimens only when the section interested the connection between the vestibular nerve and the brain stem and therefore contained some neurons belonging to the latter

The problem of the histochemical properties of the ganglionic nerve fibres was not solved As shown by Iurato et al (1971*a*) and Pannese

[1] According to most of the Authors in this paper the rm dendrite is used to refer to the process of the ochlear and vestibular ganglia neurons directed peri herally and the term axon is used to refer to the pro ss d rected centrally

et al (1974) reliable results can be obtained at an electron microscopic level only with serial sections Since this was not done in this study, no conclusions could be drawn Moreover the thick sections cut by the tissue chopper did not permit the identification of the intraganglionic spiral bundle which was found definitely positive by Nomura & Schuknecht (1965)

Since AChE activity was demonstrated in the neuronal bodies of cochlear and vestibular ganglion cells but not in the peripheral (Iurato et al, 1970, 1971*a*, 1971*b*) nor in the central (McDonald & Rasmussen, 1971) terminals of these neurons, the role of this enzymatic activity remains unclear It must be taken into account that in several zoological species AChE activity has been definitely demonstrated in spinal ganglion neurons which are regarded as non cholinergic in nature (Eccles, 1964, Bremer, 1953, Feldberg, 1954) On the role of AChE activity in the neuronal bodies of cochlear and vestibular cells the following hypotheses can be put forward

(1) The cochlear and vestibular ganglion cells are non cholinergic neurons and AChE activity has only a "vestigial" significance (Koelle, 1955, Feldberg, 1957)

(2) AChE activity in these neurons plays the universal role of restoring membrane permeabil- to the resting state in the conducting cells 1959, 1963)

AChE plays an intermediate part in the of the actual transmitter, whether it is ACh or not (Burn & Rand, 1959, 1965, Koelle, 1963, Eränkö, 1967)

On the basis of the information available up to now it is not possible to determine which of the above-mentioned hypotheses is more likely to be the correct one

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Der histochemische Nachweis der Acetylcholinesterase Aktivität in cochlearen und vestibularen Ganglienzellen des Innenohres von Chinchillas wurde unter Verwe der Karnovskyschen Methode durchgefuhrt. Das

produkt am Axolemm sichtbar Es fehlte in Satel und Schwannschen Zellen Kontrollversuche die Spezifitat der Reaktion auf Acetylchol Aktivitat

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THE VESTIBULAR AQUEDUCT IN PATIENTS WITH MENIÈRE'S DISEASE

A Tomographic and Clinical Investigation

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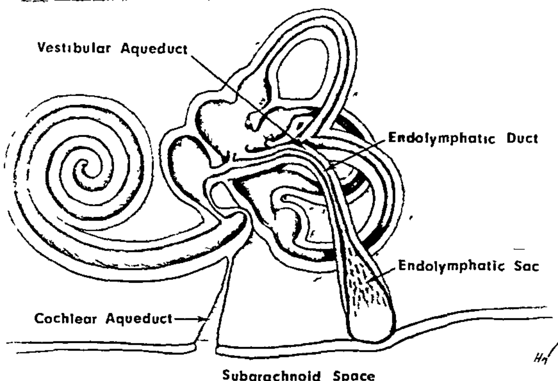
Abstract The vestibular aqueduct was identified on tomograms *in all healthy subjects In patients with severe* Menière s disease the visibility was affected The aqueduct was visible in 65 % of the non-diseased ears and in 59 % of the diseased ears of patients with unilateral Meniere s disease, whereas the corresponding figure for bilaterally diseased ears was only 53 % Longstanding disease can impair the visibility, while it does not seem to be affected by advanced age *per se* No obvious relationship was found between the visibility on tomograms and the results of hearing tests and caloric tests The vestibular aqueduct is shorter in patients with Menière s disease than in healthy subjects The pneumatization of the pyramid, which varies in normal individuals as well as in patients with Menière s disease, influences the length of the aqueduct The longest aqueducts in healthy subjects were found in those with large cell pneumatization in vicinity of the aqueduct Most patients with Meniere s lacked pneumatization of the pyramid and the aqueducts were found amongst them The aqueduct and the adjacent para vestibular iculus have been demonstrated by means of plastic moulding Minute communicating channels probably housing parts of the blood supply to the endolymphatic *duct and sac, have been revealed The para vestibular* canaliculus which lies at the borderline of the tomographically demonstrable structures could be discerned in the tomograms of 10 of the 32 ears of healthy individuals and 10 of the 86 ears of patients with Meniere s disease

A well functioning endolymphatic duct and sac is said to be a pre-requisite for normal labyrinthine function Experimental studies have indicated that the main function of the endolymphatic sac is to act as a resorptive and defensive mechanism for the inner ear (Lund 1965) Functional ablation of the sac lea endolymphatic hydrops (Kimura & Schukn 1965, Kimura, 1967, Schuknecht et al 1 The rapidity of the onset and severity of hyd following ablation of the endolymphatic varies with the species (Yuen & Schukn 1972)

Structural abnormalities of this duct and impairing their resorptive function, are tho to be a major factor in the pathogenes Menière's disease (Shambaugh et al, 1 Resorption of endolymph might be distu through a deficiency of its circulation in duct, due to narrowing of the vestibular a duct This latter can be caused by the forma of subepithelial concrements, which in time be transformed to bone, resulting in chr retention hydrops" in the inner ear (Wittma 1956) In Menière's disease exostosis like b of lamellar bone on the walls of the aque have been described by Zechner & Alt (1969) and Zechner (1973) Complete b plugging in sporadic cases has been repo (Clemis & Valvassori, 1968)

The function of the duct and sac may be i fered with by subepithelial avascular fib producing ischaemia of the sac wall (S baugh et al, 1969) Such perisaccular fib was first described by Hallpike & Cairns (1 in patients with Menière's disease as well a normal subjects Zechner & Altmann (1

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1 Schematic drawing of the endolymphatic and peri
iphatic systems and their relation to the subarachnoid
ice

ıwever, only observed such fibrosis in speci-
ens from persons with Menière's disease
Results contradictory to the above-mentioned
ıdings have been reported in two recent papers
ıus Yuen & Schuknecht (1972), after histolo
cal examination of temporal bones from 19
tients with Menière's disease, concluded that
e vestibular aqueducts in Menière's patients
e not different in calibre from those of nor-
al ears The endolymphatic ducts, on the other
nd, were found to be narrower than those in
e control group Gussen (1973), describing one
igle patient, stated that the endolymphatic duct
d intermediate portion of the sac of the dis-
sed side had a rather straight course to the
sterior fossa with virtually no lateral curving
herwise it appeared normal, with a small
ıount of loose tissue about its intermediate
rtion The duct and sac on the opposite,
althy side had the same appearance

The aim of our investigation was to study the tomographic visibility and length of the vestibular aqueduct in (*a*) a group of healthy subjects and (*b*) a group of patients with very disabling Menière s disease The visibility was correlated to clinical data in an attempt to assess the diagnostic value of the radiographic examination The pneumatization of the aqueductal region was correlated to anatomic and clinical findings In addition an attempt was made to find the para vestibular canaliculus, this has not previously been identified radiographically either in normal subjects or in patients

General morphology and previous tomographic investigations

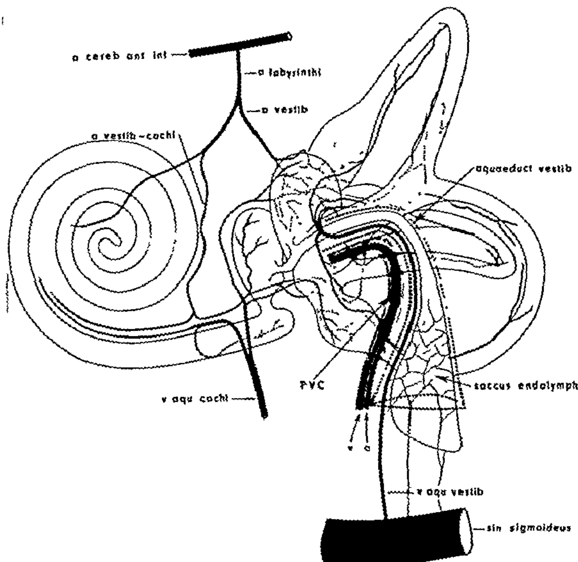
The vestibular aqueduct originates from the medial wall of the vestibule passing through the otic capsule immediately in front of and somewhat medial to the *crus commune* (Figs 1 and



Fig 2 Temporal bone preparation demonstrating the courses of the vestibular and cochlear aqueducts (black-stained) The proximal portion of the vestibular aqueduct parallels the crus commune (*CC*) of the superior and ...ior semicircular canals The peripheral portion ...s a flat, triangular widening A small bony ridge is left to mark the external aperture of the vestibula... duct (*right arrow*) The distinct round opening (... corresponds to the canal for the posterior am... nerve The left arrow points at the external ope... the cochlear aqueduct *MAI*, internal acoustic ... *SS* sigmoid sulcus

...It then curves (Figs 2 and 3), forming a ...ore or less pronounced angle on its course to the external aperture on the cerebellar surface of the petrous bone (Bast & Anson, 1949; Wilbrand et al, 1974) The external opening, which in most instances can be recognized about 10 mm posterolateral to the *porus acusticus internus* and 10 mm inferior to the *sulcus petrosus superior* (Anson, 1965, Anson et al, 1968) may show great individual variations It can be more or less sheltered by a bony ridge (Figs 2 and 7) The endolymphatic sac is partly housed in the vestibular aqueduct The *pars rugosa* of the sac occupies the flat, triangular distal part, while the extraosseous portion lies intradurally close to the sigmoid sinus (Bast & Anson, 1949) The ratio of the intra-osseous to the intradural part of the sac in man varies with the degree of ...matization of the pyramid (Wittmaack ... Rich pneumatization is said to be accomp... by a long intraosseous and a short intra... part Sparse or absent pneumatization, o... other hand, should mean a short intraos... and a long intradural part. These ana... variations may contribute to the difficulti... ported in identifying the sac at drainage o... tions (House, 1964)

The arterial blood supply of the vest... aqueduct and its content is still unclear (F... Bast & Anson (1949) have described ... branches from the posterior meningeal a... supplying the dura around the endolym... sac, and accompanying the duct for a ... distance. Small veins form a plexus arou...



g 3 General survey of the vascular supply of the vestibular part of the human inner ear The cochlear vascularization has been purposely neglected The arterial supply takes place through the vestibular artery and by vestibular branches of the vestibulocochlear artery The venous drainage occurs partly by branches to the vein in the cochlear aqueduct and partly by the vein in the vestibular aqueduct ending in the sigmoid sinus The shaded area, *P V C.*, represents the para vestibular canaliculus housing an artery and vein

distal widening part of the aqueduct The venous blood from the non-acoustic labyrinth is mainly collected to the vein of the vestibular aqueduct (Beck 1965) This vein receives numerous small veins from the plexus around the endolymphatic duct before it finally drains into the sigmoid sinus (Bast & Anson, 1949)

Tomographic reproductions and descriptions of the aqueduct have been published by Clemis & Valvassori (1968), Wilbrand (1971), Brünner & Pedersen (1971), Sesana et al (1971) and Bisch & Barrionuevo (1972) Wilbrand et al (1974) found that in tomograms of temporal bone specimens the entire aqueduct was visible in 51 % of the cases On subsequent microdissection the aqueduct was identified in all specimens The inability to visualize all aqueducts tomographically was considered to be mainly due to

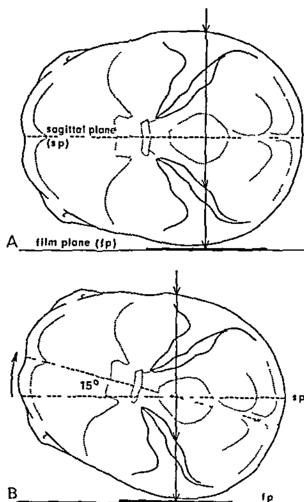


Fig 4 Schematic drawing of tomographic positioning applied in this investigation (A) True lateral projection, Slight elevation of the face (15°) from the true lateral ion

quate positioning of the specimens In a clinical study Clemis & Valvassori (1968) reported that in patients with hearing loss of the conductive type they were able to identify the aqueduct on tomograms in more than 90 %, while in 28 out of 35 patients with Meniere's disease it could not be identified On the basis of these findings Arenberg et al (1970) have recommended decompression surgery of the endolymphatic sac only in those patients in whom the vestibular aqueduct has been found to be open on tomography

The para-vestibular canaliculus

In 1971, Ogura & Clemis discovered on microdissection a tiny bony canal which they designated the para-vestibular canaliculus It w after osmic acid fixation in 15 out of 23 specimens, running from the vestibule posterior cranial fossa, in some cases proximity to the vestibular aqueduct, others a certain distance away The can contained an artery and a vein, which the thought to constitute the blood supply endolymphatic sac They also attribu canaliculus the function of a pressure eq valve between the perilymphatic spa posterior fossa Wilbrand et al (197 studied 35 human temporal bone were able to identify the canaliculus on grams in seven of the specimens, on sub microdissection the osmic acid stained culus was verified in all specimens D canaliculi were observed as well as va in the course The morphology and func the blood vessels lodged within the para ular canaliculus have not yet been evalua

MATERIAL AND METHODS

The investigation was made on

(*a*) Twenty healthy persons between 15 years of age (mean age 33 years) Bo were tomographed in 12 subjects and o in the remaining eight, making a total of examined

(*b*) Forty-three patients (12 women and 3 with severe Meniere's disease They w tween 22 and 76 years old (mean age 48 According to the hearing tests the disea unilateral in 33 and bilateral in 10 patien ears (86) were tomographed The age patients at the onset of the disease was years (mean age 41 years) The duration disease at the time of the investigation between 1 and 30 years (mean 7 7 years pure tone threshold of the diseased ears from 18 to 100 dB (mean 53 dB), the discr tion scores for the same ears being 0- (mean 61 %) The caloric response assessed electronystagmograms was reduced in 5 the patients with unilateral disease, the av difference in excitability (maximum speed

able I *The visibility of the vestibular aqueduct in tomograms, expressed as the percentage number of ears with visible aqueducts*

	Patients with Menière s disease (86 ears)		
	Unilaterally diseased		
Healthy subjects (32 ears)	Non diseased ear	Diseased ear	Bilaterally diseased
100%	65%	59%	53%

ow nystagmus phase) was 32%. Among the ,l patients with bilateral disease the caloric response was abnormal in eight and normal in three

Tomography was carried out with the Philips-Massiot Polytome, using its hypocycloidal movement at a magnification of 1 3 A focal size of 3 mm was used The exposure data were 55- kV 50 mA and 11 6 sec (double hypocycloidal movement) The radiographic system consisted of a cassette with high definition screens and a RPL film The technique thus corresponded to that used in previous investigations (Sandstrom & Wilbrand 1971, Dahlin et al, 1973 Wilbrand et al, 1974, Wilbrand, 1974)

On the basis of earlier experience (Wilbrand et al 1974) the subjects were tomographed (1) in the true lateral position and (2) in a position with the face elevated 10–20° from the true lateral position (Fig 4) In no case was correction of the positioning needed in order to visualize the proximal portion of the vestibular aqueduct A distance between the cuts of 0 5 mm was used so as to guarantee overlapping of the information content of every cut

Evaluation of the individual tomograms implied (1) repeated checks of the visibility of the entire aqueduct, (2) measurement of the proximal and peripheral portions of the aqueduct and (3) grading of the pneumatization in the vicinity of the aqueduct The tomograms were viewed under optimal conditions (Rohler 1967) Apart from magnifying glasses no further image quality enhancing procedure was employed The measurements were performed by means of a vernier caliper (division 1/10 mm)

The material was processed by an IBM 370 Model 155 computer at the Data Center of the University of Uppsala (UDAC)

RESULTS

1 The vestibular aqueduct was visible on the tomograms of all healthy subjects (32 ears) (Table I, Fig 5) The mean total length from the internal or vestibular aperture to the external aperture was 8 7 mm (6 1–13 5 mm) (Table II)

2 In patients with unilateral Meniere's disease (66 ears), the aqueducts were visible on the non-diseased side in 65% and on the diseased side in 59% of the ears In bilateral cases this figure was reduced to 53% of the ears (Table I)

3 The total length of the aqueduct in unilateral cases of Meniere's disease (Table II) was 5 1–11 7 mm (mean 7 3 mm) on the non-diseased side and 4 6–10 2 mm (mean 6 8 mm) on the diseased side In patients with bilateral disease the total length varied between 4 3 and 9 8 mm (mean 6 7 mm)

4 The tomographic visibility of the aqueducts was correlated to several clinical characteristics (Table III) When comparing the mean values, no correlations were found between visibility/non visibility, on the one hand, and age, pure tone threshold, discrimination score or caloric response, on the other The impairment of the inner ear function in the patients with Meniere s disease, taken as a whole, thus seemed to be of

Table II *The length of the vestibular aqueduct, measured on tomograms*

	Patients with Meniere s disease		
	Unilaterally diseased		
Healthy subjects (32 ears)	Non diseased ear (26 ears)	Diseased ear (21 ears)	Both ears affected (14 ears)
8 7 mm (6 1–13 5)	7 3 mm (5 1 11 7)	6 8 mm (4 6–10 2)	6 7 mm (4 3–9 8)











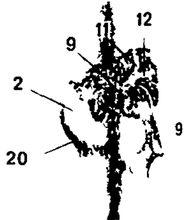


Fig 5 Tomograms of three healthy subjects demonstrating variations in the pneumatization of the pyramid The longest aqueduct is seen in the most pneumatized pyramid (uppermost picture) Notations in the schematic drawings *2* tympanic space *9* vestibule *9d* v aqueduct *11* superior semicircular canal *1'* l semicircular canal *20* tympanic ring, *P* pneuma

III *Tomographic visibility of the vestibular aqueduct in patients with Meniere s disease, correlated to various clinical factors*

aterial	Variable mean values	Vestibular aqueduct Visible	Non visible	)
ll ears	Age years	47 8 (*n* 47)	49 7 (*n* 37)[a]	0 86
	Age at onset years	41 2 (*n* 47)	41 1 (*n* 32)	0 97
	Pure tone threshold dB	28 3 (*n* 47)	35 0 (*n* 37)	0 97
	Discrimination score %	79 1 (*n* 47)	73 9 (*n* 32)	0 71
	Caloric response diff in %	*26 1 (n 47)*	*34 1 (n 37)*	*1 3*
iseased ears only	Duration of disease years	6 7 (*n* 27)	10 7 (*n* 26)	1 96[b]

Seven ears excluded for technical reasons
ignificant *P* 95%

same extent irrespective of the radiographic sibility of the vestibular aqueduct A significant lation ($P-95\%$) was noted between the isibility and the duration of the disease, when nly diseased ears were taken into account the uration being longer in patients with a non isible aqueduct

5 The pneumatization of the pyramid obser-ed on tomograms varied both in healthy sub ects and in patients with Meniere's disease On he basis of observations on the healthy subjects hree main groups could be discerned designated 1 2 and 3 (Figs 5 and 6) Group 1 consisted of ars with large cell pneumatization in the vicinity f the aqueduct and group 3 ears without pneu atization whereas group 2 included ears with arying types of pneumatization mostly of mall cells The data are presented in Table IV, hich reveals an abnormal pneumatization pat rn in Meniere's disease, characterized by a high equency of non pneumatized pyramids

The total lengths of the aqueducts obtained from the tomograms in relation to the degree of pneumatization are presented in Table V In group 1 with large cell pneumatization of the pyramid the aqueducts were longer than in group 3 with no pneumatization The difference in length was mainly due to a long peripheral portion in the former group The mean length of 10 3 mm in healthy subjects of group 1 (Table V) exceeded the mean value for the ears of all healthy subjects which was 8 7 mm (Table II) The shortest aqueducts were found in those patients with Meniere s disease with no peri aqueductal pneumatization

able IV *The pneumatization of the petrous portion of the temporal bone as assessed by tomography (figures indicate the number of ears)*

	Group 1 Large cell *pneumatization*	Group 2 Small cell *pneumatization*	Group 3 No pneu *matization*
Normal subjects (32 ears)	11 (34%)	14 (44%)	7 (22%)
Menière s disease (86 ears)	0	28 (26%)	58 (74%)

6 No significant correlation was found between the different grades of pneumatization and the visibility of the aqueduct in patients with Meniere s disease

7 The external aperture of the aqueducts varied in shape and width depending upon the *pneumatization Bony ridges sheltering the* opening were found in large cell pneumatized pyramids In non pneumatized bones on the other hand the aqueducts sometimes opened out freely (Figs 5 and 7)

8 The para vestibular canaliculus was visible on the tomograms of 10 out of 32 healthy ears With reference to the pneumatization of the pyramids four of these ears belonged to group 1 and three to each of groups 2 and 3 The canaliculus was observed in 10 of the 86 ears of patients with Menière s disease This structure was also demonstrated by means of plastic filling in temporal bone specimens (Fig 8)

Table V *Pneumatization and length of the vestibular aqueduct in healthy subjects and in p... with Meniere's disease*

Grading of pneumatization of the pyramid	Healthy subjects Total length of aqueduct (32 ears)	Patients with Menière s disease Total length of aqueduct (86 ears)
Group 1—large cell pneumatization	10 3 mm (7 2–13 5) 11 ears	No patients
Group 2—small cell pneumatization	8 2 mm (6 1 9 9) 14 ears	7 7 mm (5 9–11 7) 28 ears
Group 3—no pneumatization	7 4 mm (6 7–8 1) 7 ears	6 9 mm (4 3–10 2) 58 ears

DISCUSSION

There is no doubt about the existence of definite differences in the radiographic visibility of the vestibular aqueduct when comparing healthy subjects and patients with Meniere s disease While the aqueduct was visible in its entire length in all healthy subjects, it was observed in only 59 % of the diseased ears in patients In bilateral cases this figure was reduced to 53 %

The mean whole length of the aqueduct in normal persons, measured on the tomograms in this investigation, was 8 7 mm (range 6 1–13 5 mm) This figure may be compared with earlier values obtained by our group (Wilbrand et al, 1974) in other types of studies, namely 8 1 mm (range 5 7–10 9 mm) in measurements on temporal bone specimens and 8 5 mm (range 6 1–... 6 mm) in direct measurements on dissected ...imens The latter values agree well with ...ts presented by Anson et al (1965) and ...gura & Clemis (1972)

The length of the entire aqueduct, measured on tomograms was found to be shorter in ears affected by Meniere's disease than in no... ears On the whole, the shortest aqueducts ... noted in patients with bilateral Meniere s d... The mean length for that group was 6 7 (range 4 3–9 8 mm), which may be com... with the length in healthy subjects, viz. 8 7 (range 6 1–13 5 mm) This difference is s... cally significant at the 99 % level

Up to now only one major study has ... published in which the radiographic find... have been compared with the inner ear fu... Thus Clemis & Valvassori (1968) have re... a high correlation between abnormal he... and a non visible aqueduct We have com... the pure tone threshold, the discrimination ... and the caloric response with the radio... results without finding any obvious relatio... (Table III) In other words an apparent h... loss, for example, is not necessarily accom... by a non visible aqueduct We agree with t... opinion of Arenberg and others that any ... of drainage operations on the sac in pa... with a radiographically non visible aqueduct ... probably meaningless because they would n... duce an inner ear hydrops House & Owens (1... in a long term follow up on patients with M... ère's disease treated with subarachnoid ... reported failures in 38 % which they attrib... among other things, to closure of the ... lymphatic duct Preoperative tomography of ... vestibular aqueduct therefore seems to be ... importance when planning operations on ... sac

The relationship between the radiograph... results and the age of the patient was also s...



Fig 6 Different types of peri aqueductal pneumatization (1) large-cell pneumatization in the vicinity of the aqueduct (2) small-cell pneumatization in the vicinity of the aqueduct (3) absence of air cells *P* = pneumatization *ae* external aperture of the vestibular aqueduct



7 A B Different shapes of the external aperture of vestibular aqueduct (arrows with black outlines) te the paucity of air cells in the lower part of the pyra l and the mastoid portion of the left specimen (A) h a very small external aperture of the aqueduct, in contrast to the extensive pneumatization of the pyramid and mastoid portion of the right specimen with a large external aperture of the aqueduct (B) *MAI* internal acoustic meatus *SS* sigmoid sulcus



Fig 8 Plastic mould of a human inner ear demonstrating he course of the vestibular aqueduct (white arrow) and adjacent para-vestibular canaliculus (two arrows white outlines) from the medial wall of the vestibule , the cerebellar surface of the pyramid The bend of the estibular aqueduct lies next to the tip of the white arrow The para-vestibular canaliculus runs close to the posterior semicircular canal (*PSC*) The upper facial ... marked with an asterisk Note the minute vascular ... munications between the aqueduct and the para ves... canaliculus, which in its distal portion is split ... branches (the arrow furthest to the left) *MAI* ... acoustic meatus, *SSC*, superior semicircular canal *L*... lateral semicircular canal

and no correlations were found (Table III) High age was not reflected by a non-visible vestibular aqueduct This was surprising in view of the findings of Wittmaack (1956) and Zechner (1973) of bony obstruction of the aqueduct in elderly subjects, which might be expected to make the radiographic identification more difficult It must be pointed out, that no very old persons were included in our material, where the age of the normal subjects ranged from 15–49 years (mean 33 years) and that of the patients with Meniere's disease from 27–76 years (mean 48 years)

Besides differences in the aqueductal le... between normal subjects and patients with M... ère's disease, there are also obvious differ... in the degree of pneumatization in these ... groups Large cell pneumatization in the ... of the aqueduct is accompanied by long a... ducts As patients with Menière's disease ... ly lack pneumatization of the pyramid, t... aqueducts will be short Presuming that p... matization of the pyramids is regulated by ... genital factors, it is possible that failure of ... and subsequent shortness of the aqueducts ... predispose to Menière's disease

has been found that the para-vestibular ılus, which is sometimes visible on tomo- of normal individuals as well as of patients Meniere's disease, can be demonstrated by ıs of plastic moulds (Wilbrand & Stahle,) The method will be described in detail in rthcoming paper (Wilbrand, 1974) Gener- it runs adjacent to the vestibular aqueduct 8) Distal branching occurs Several minute ›ns between the canaliculus and the ›duct have been disclosed, housing small d vessels Their intimate topographical ıonship to the vestibular aqueduct contri- s to the complexity of the morphological ›rs which with all probability are linked to pathogenesis of Meniere's disease

ZUSAMMENFASSUNG

Aquaeductus vestibuli liess sich in Tomogrammen nder Personen immer darstellen Bei Patienten, die :hwerem Morbus Meniere leiden ist die Darstellungs lichkeit beeinträchtigt Der Aquädukt war bei ein g Erkrankten im gesunden Ohr in 65% und im ıken Ohr in 59% der Falle sichtbar Die entsprechende l bei doppelseitig Erkrankten war nur 53% Die nkheitsdauer hat einen Einfluss auf die Darstellungs- :lichkeit, welche jedoch in keinem Zusammenhang hohem Lebensalter steht Zwischen der tomographi n Darstellbarkeit und den Ergebnissen der Gehor ›rsuchung und der kalorischen Probe wurde kein tbarer Zusammenhang gefunden

ler Aquaeductus vestibuli ist bei Menierepatienten zer als bei gesunden Personen Die Pneumatisation Pyramide, welche bei Gesunden wie auch bei Meniere- enten gewisse Schwankungen aufweist, hat einen Ein- s auf die Länge des Aquadukten Die langsten Aqua- te wurden bei Gesunden gefunden, welche eine aus ›nnte Pneumatisation in der direkten Umgebung des ıadukten besassen. Die Mehrzahl der Menierepatien- ›hatte kaum pneumatisierte Pyramiden, und die kur- ›en Aquadukte wurden auch bei ihnen gefunden Mit tischen Gussmodellen ist der Aquaeductus vestibuli der ihn begleitende paravestibuläre Canaliculus ab- ›ildet worden Winzige Verbindungskanalchen die ›rscheinlich Teile der Blutversorgung des Ductus und us endolymphaticus beherbergen, sind veranschau worden

›er paravestibulare Canaliculus liegt im Grenzbereich tomographisch Darstellbaren und konnte in Tomo- nmen von 10 der 32 Ohren gesunder Personen dar ›ellt werden, jedoch nur in 10 Fallen von 86 Ohren ›A Menièrekranken Diese Struktur durfte fur den abolismus im Saccus endolymphaticus von Bedeutung › und damit einen Einfluss auf die Funktion des Innen ›rs ausuben

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A THREE DIMENSIONAL MODEL OF THE CAT LABYRINTH

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ract A method is offered for the construction of a : dimensional plexiglas model of the cat vestibular organ

:nowledge of both the anatomy and orien on of the various structures comprising the ıbular end organ is essential for an under- ıding of the physiology of the vestibular :em

ι three dimensional plexiglas model of the yrinth, magnified sufficiently, would aid in visualization of the vestibular apparatus rough the use of plexiglas, anatomical rela- nships between superficial as well as interior uctures can be examined The purpose of this per is to present the method for construction this model

METHOD

mporal bones of a cat were removed, fixed th formalin, and embedded in celloiden Sec ıns were cut at 20 μm, mounted, and stained th hemotoxylin and eosin The bone was ıented for cutting to assure that the macula of e utricle was approximately in the horizontal ane This ensured that the model when as- mbled would show the desired structures as ey existed in the head erect position of the cat ıe bone may be oriented in any other position, ıt this would cause the assembled model to be ted in a position other than head erect

Each fifth section was used for the construction the model with the exception of those areas where larger intervals were sufficient for the needed detail (i e , from the level of the common crus to the apex of the vertical semicircular canals) A micro projector (Bausch & Lomb, tri simplex) was used to magnify the slide onto a clear plexiglas square measuring $6'' \times 6'' \times 1/8''$ A $\times 20$ magnification factor was determined from the ratio of the size of the anatomical structures on the plexiglas square to their size on the slide This factor limited the thickness of the plexiglas sheet, as any thickness greater than the distance between corresponding surfaces of successive sections times the magnification factor would cause distortion of the three dimensionality of the model along this axis

The bony and membranous labyrinths were traced onto each square using a LeRoy writing set with indelible ink of different colors The cristaes of the semicircular canals and maculaes of the otolith organs were distinguished from other structures by a thicker inked line Precise positioning of the projected image on each square was necessary for alignment of the desired structures in the assembled cube The use of specific landmarks, such as mastoid air spaces, was helpful for this

The model was assembled with two 1/4" bolts The separation between the plexiglas squares was accomplished with tubular plexiglas spacers These spacers were cut to a thickness that would make the distance between corresponding surfaces of each successive plexiglas square equal to the distance between the cor-

responding temporal bone sections times the magnification factor

In order to provide clarity, the picture (Fig 1) was taken with the model submerged in water Because the optical densities of water and plexiglas are similar, the three dimensionality of the model becomes evident Knowing that a two dimensional picture does not allow for a three dimensional interpretation, the photograph was taken with a small depth of field hoping to give the impression of a third axis

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The technical assistance of the Temporal Bone La[illegible]tory, Department of Surgery, Division of Otolaryng[illegible] University of Florida Gainesville Florida, is apprec[illegible]

ZUSAMMENFASSUNG

Eine Methode zur Herstellung eines Plexiglasmodel[illegible] vestibularen Endorgans der Katze wird vorgeleg[illegible]

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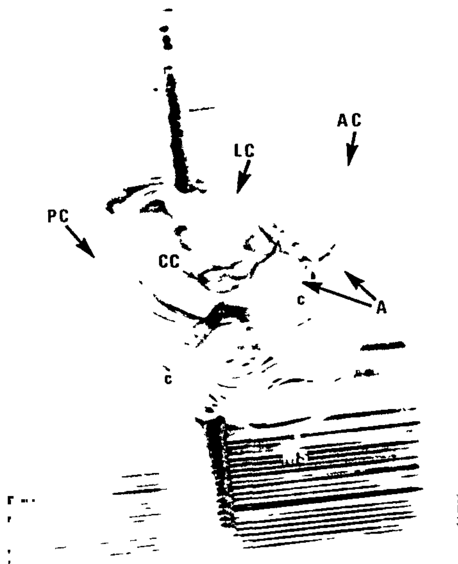


Fig 1 Three dimensional plexiglas model of the right labyrinth of a cat Structures from lateral and inferior to medial and superior The bony and membranous are shown in yellow and black respectively

LC	lateral semicircular canal	MS	macula of the saccule
AC	anterior semicircular canal	CC	common crus
PC	posterior semicircular canal	A	ampulla
MU	macula of the utricle	c	cristae

(Photographic technique see text)

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EFFECTS OF D-TUBOCURARINE ON THE AMPULLAR RECEPTORS OF THE FROG

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Abstract In order to better define the action of D Tubocurarine on the ampullar receptors in frog vestibular apparatus the drug was applied to isolated open semicircular canals as well as to the entire undissected labyrinths Curare was able to depress or abolish according to the doses employed (50-200 μg/ml) both the resting discharge of the ampullar receptors and their responses to mechanical or electrical stimulations only when it was administered to the isolated open semicircular canals contrariwise almost no effect was detectable when curare was administered to undissected preparations The action of this drug develops earlier on the sensory units responsible for spikes of higher amplitude than on those responsible for medium and lower spikes this effect may be interpreted as an enhancement of the threshold extended to the whole receptor population comprising the crista ampullaris. Curare blockade of the mechanical responsiveness in ampullar receptors is fully reversible in about 15 min after withdrawal of the drug while both resting discharge and electrical responsiveness are much more rapidly restored and even enhanced in excess of normal values after washing The site and the possible mechanism of action of D Tubocurarine on ampullary receptors are discussed

Some observations indicate that curare is able to interfere in the mechanism of sensory processing taking place in labyrinthine receptors

In preliminary experiments, Dohlman (1967) observed that curare can depress sensory discharge in semicircular canals Kuiper (1956) found that the curare like substance Flaxedil depresses the microphonic effect of the lateral line organ and he interpreted this action of the drug as being due to the impairment of a cholinergic process located in the mechano-sensitive membrane of the hair cells

The presence of a cholinergic step in the activation of labyrinthine receptors is, however, a debatable point On the basis of histochemical evidence Lowenstein (1967) and Vinnikov (1969) have suggested that acetylcholine might be the transmitter at the cytoneural junctions of ampullar receptors, this view has however been disproved by the data of Osborne & Thornhill (1972) and Thornhill (1972) which point to a monoamine as being the transmitter at these junctions

On the other hand, Ireland & Farkashidy (1961) and Hilding & Wersall (1962) claim that acetylcholine may mediate the inhibition of ampullar receptors at the junctions of the efferent fibres

The present experiments were devised with a view to a better definition of the action of D Tubocurarine in the sensory apparatus of semicircular canals and to gain some insight into the processes which take place in the ampullar receptors

Curare was applied to isolated open semicircular canals as well as to the entire undissected labyrinth of the frog In the former condition the drug most probably contacts both poles of the sensory cells rapidly In the second, curare very likely reaches the outer pole of the sensory cells with ease but its penetration into the endolymphatic space is likely to be hindered or even

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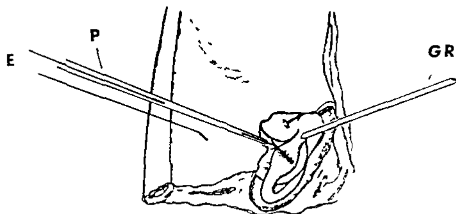


Fig 2 Schematic representation of the experimental set-up for stimulating and recording of action potentials in undissected preparations *P*, leading-off pipette, *E*, Ag/AgCl electrodes, *GR*, stimulating glass rod

byrinth The ampullar nerve was carefully dissected

Mechanical stimulation of the ampullar receptors was performed by means of a thin glass rod placed against the wall of the canal near its opening into the utricle (Fig 2) The glass rod was driven by the moving coil of the small loudspeaker and its step displacements were so chosen as to evoke discharges similar to those produced in isolated preparations

In all the experiments the afferent discharge was picked up from fibre twigs dissected from the ampullar nerve, which were sucked into a glass pipette provided with Ag/AgCl electrodes Nerve action potentials were amplified in a c 1000 ×, monitored on a CRO and recorded on FM magnetic tape The spikes were counted electronically and classified into two groups by means of an amplitude discriminator, i e >150 μV (higher voltage spikes) and ≤150 μV (medium and lower voltage spikes)

The responses of the ampullar receptors to mechanical and electrical stimuli were recorded before and during the exposure to curare for 15 min and also after withdrawal of the drug D-Tubocurarine was added to the bath in such amounts as to reach final concentrations ranging between 50 and 200 μg/ml

At least 5 similar experiments from different preparations were carried out in each experimental condition Averaging and processing of data were performed by means of a CAT 400 C interfaced with a PDP 8/L computer

RESULTS

Fig 3 shows an example of the tracings obtained from experiments in which D-Tubocurarine was applied to the intact labyrinth It may be noted that even at the higher doses tested (200 μg/ml) the drug affected neither the resting discharge of the receptors nor their response to mechanical stimulation to any substantial degree

On the contrary, D-Tubocurarine produced a marked depression of the receptor discharges in isolated open canals Its action was already evident at a concentration of 50 μg/ml and increased with the dose until, at 150–200 μg/ml,



Fig 3 Discharges of ampullar receptors in response to mechanical stimulation in an undissected preparation *Upper trace* control *Lower trace* after exposure to D Tubocurarine (200 μg/ml) for 15 min

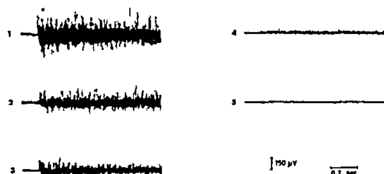


Fig 4 Discharges of ampullar rec
in response to a mechanical stimul
medium strength recorded 10 m
exposure to D Tubocurarine at d
concentrations
1 control *2 3 4 5* 50 100 150, 2
of Tubocurarine/ml of Tyrode solu

both resting and evoked discharges were completely suppressed The blockade by D Tubocurarine develops in a few minutes and reaches a steady level in about 10–15 min

As illustrated by the tracings in Fig 4, curare block becomes established in the receptors responsible for spikes of higher voltage even at the lowest dose employed, whereas a complete block of the receptors giving rise to spikes of medium and lower voltage was produced by D Tubocurarine only at the highest doses (150–200 µg/ml) However, after the action of moderate doses of the drug (Fig 5) the units which no longer responded to weak mechanical stimuli could be reactivated by increasing the stimulation strength, thus revealing that curare had enhanced the threshold of these units

The dose-effect relationship may be ded from the graphs in Fig 6 which refer to peak discharge frequency of the responses mechanical stimulus of medium strength. S rate graphs are drawn for the lower and me voltage (A) and the higher voltage spikes (B

Fig 6C also illustrates the action of D-T curarine on the resting discharge, which wa a rule evaluated over the 30 sec preceding st lation It may be seen from the graph in figure that blockade of the resting disch requires concentrations of the drug much h than those able to depress the response of receptors to mechanical stimuli

The effects of D Tubocurarine are ra reversed after washing This is clearly indic in the graphs in Fig 6, which evidence a c

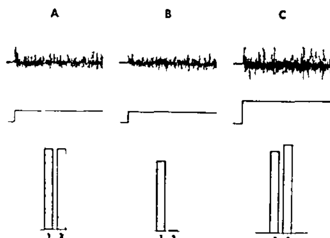
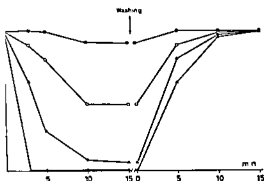
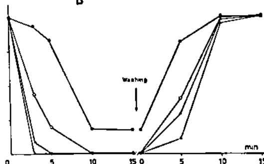
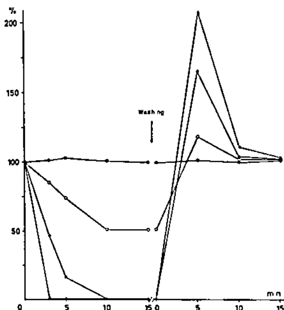


Fig 5 Responses to weak and to strong mechanical stimuli before and after exposure to a moderate dose of D Tubocurarine (50 µg ml) (A) Response to the weak stimulus in plain Tyrode (B) Response to the same stimulus 10 min after addition of D Tubocurarine (C) Response to a stimulus of doubled intensity 15 min after addition of D Tubocurarine *Upper part* examples of the records *Middle part* stimulus intensity expressed in placement of the microsyringe plunger *Lower part* histograms refer to the peak responses (mean f experiments) expressed as percentages of norm medium lower voltage units (1) and for higher vo units (2)







g 6 Time course of the effects of D Tubocurarine at fferent concentrations *Left side of the picture* peak sponses to mechanical stimulation of medium intensity the lower medium voltage units (A) and of the higher ltage units (B) *Right side* mean frequency of the sting discharge evaluated before stimulation (C) Washing at the break in the abscissae The values are expressed as percentages of normal Means from 5 experiments ● D Tubocurarine 50 μg/ml ○ D Tubocurarine 100 μg/ml ▲ D Tubocurarine 150 μg/ml △ D Tubocurarine 200 μg/ml

lete recovery of receptor responsiveness, 10–15 in after withdrawal of the drug, irrespective f its concentration It may be noted (Fig 6C) iat the resting discharge recovers much earlier ian the response to mechanical stimuli, being ready complete within 2–3 min after washing urthermore, there is a period of 5–10 min, uring which the resting discharge of the re ptors is markedly enhanced to above its noral level this enhancement is greater the larger ie dose of curare, and therefore the deeper the epression previously suffered by the receptors

Some examples of tracings from the experi ients on open canals in which both mechanical nd electrical stimuli were employed are reorted in Fig 7 A quantitative comparison beween the responses to both electrical and iechanical stimulation as obtained from all these experiments can be made from the graphs in Fig 8 It can be seen that at a dose of curare capable of abolishing the responses to mechanical stimuli (200 μg/ml), D Tubocurarine is also able to reduce considerably the receptor discharge evoked by electrical stimuli The response to electrical stimulation, however, was at no time completely suppressed by the drug at the doses we used

The comparison between the effects of each kind of stimulation during the recovery period following withdrawal of the drug appears to be of particular interest In fact, it may be seen that the electrical excitability of the receptors becomes completely restored much earlier (2–3 min after washing) than their ability to respond to mechanical stimuli, furthermore, responses in excess of normal values to current flow are

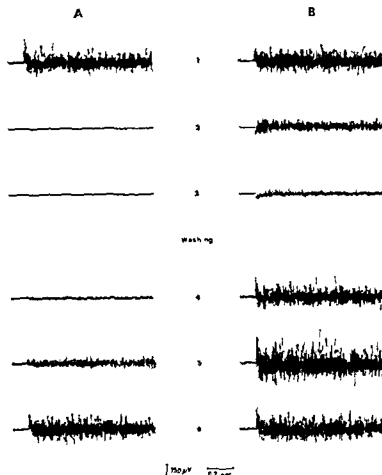


Fig 7 Comparison between the re[…] of the ampullar receptors to a me[…] (A) and to an electrical (B) stim[…] medium intensity *1* controls *2* […] *3* 10 min after D Tubocurarine 20[…] *4, 5, 6* 1, 3 and 10 min after wash[…]

observed after recovery This period of above normal responsiveness of the receptors to electrical stimulation was found to parallel the […] of their resting discharge previous-ly described The divergence between el[…] and mechanical responsiveness reaches a […]mum in the first few minutes after was[…] when the preparations appear to be com[…] insensitive to excitatory cupula deflecti[…] give above normal discharges in respo[…] electrical stimulation



Fig 8 Time course of the peak responses of the ampullar receptors to mechanical (○) and to electrical (●) stimuli of medium intensity during the exposure to D Tubocurarine 200 μg ml and after washing (at the break in the abscissae) The values are expressed as percentages of normal (means from 5 experiments)

DISCUSSION

The present experiments have clearly show[…] curare can affect the impulse discharge o[…]pullary receptors only if it is allowed to c[…] the hair-bearing surface of sensory cells C[…] did in fact prove to be ineffective on the […] labyrinth where its walls are likely s[…]tially to hinder or even prevent penetratio[…] the endolymphatic space

In isolated preparations curare acts to d[…] or completely block both the

ırge and the response of the ampullary recep-s to mechanical and electrical stimuli accord to the dose employed The depressant action the drug appears to be established through a ›gressive increase in the receptor threshold ıs mechanism may explain why curare block ⁄elops earlier in receptors responsible for kes of higher amplitude since these receptors ‑ady have a higher threshold in nature (Tag tı et al, 1974)

\s to the actual mechanism of curare action, lack of any effect of D Tubocurarine on the act labyrinth, where the drug is believed dily to contact the outer structures of the eptors, leads us to rule out any possibility competitive interaction with the transmitter at cytoneural junction of the ampullar recep-s It therefore seems more likely that curare y interfere with some step in the activation ›cesses of the sensory cells which occur at ır hair bearing pole The experiments further re revealed that spontaneous activity and chanical responsiveness of the receptors are affected by the drug to the same degree ‑ıs is suggested by the differing time course ‑ween the recovery of the receptor resting charge and that of their responsiveness to chanical stimuli, observed after withdrawal he drug During the recovery period following are block, ampullar receptors did in fact ›ve able to display a normal or even enhanced ıing activity, whereas they were still complete nsensitive to excitatory cupula deflections

n order to interpret this divergence the hypo-sis may be put forward that two curare sitive mechanisms to some extent mutually ependent may be present in the hair bearing mbrane of the sensory cells The first of these, ‑ich is more rapidly and steadily impaired by drug is likely to be involved in mechano-‑trical transduction, whereas the second, ‑ich is only affected by high doses of curare ‑l is easily reversible, may support the resting ‑harge in the receptors Obviously, both ‑hanisms must ultimately follow the same hway since they both lead to the release of ‑mical transmitter at the cytoneural junctions

Insofar as confidence may be placed in the thesis that D Tubocurarine acts as a specific acetylcholine competitor, at the doses employed, the present results may indicate that some cholinergic step is involved in the processes which take place in the hair bearing membrane of the sensory cells This view is in agreement with Kuiper's interpretation of the action of Flaxedil on the lateral line organ

The depressant action of curare on electrical responsiveness of the ampullar receptors should be discussed in the light of the aforementioned factors

Our experiments have shown that curare treatment prevents most of the excitatory effects produced by current flow through the crista ampullaris This indicates that stimulation of the ampullar receptors by current flow involves the same processes which are blocked by curare and which are thought to be located in the hair-bearing membrane of the sensory cells It follows that only the portion of the receptor response to electrical stimuli which is retained during curare block is likely to be due to direct action of the current on the endings of the afferent fibres (Löwenstein, 1955) and/or to an increased amount of chemical transmitter released at the cytoneural junctions (Del Castillo & Katz, 1954, Liley, 1956)

In this connection it is worth noting that during the clearing of curare block the recovery of the responses to electrical stimuli closely parallels that of spontaneous discharge This similarity suggests that electrical stimulation of the ampullar receptors mainly activates the same mechanism which supports their resting discharge

In order to interpret the enhancement to above normal values of the spontaneous discharge and electrical excitability in the receptors which are observed after washing of the drug it may be supposed that during complete curare block, the transmitter accumulates inside the sensory cells and that this accumulation facilitates release of the transmitter after withdrawal of the block

On the basis of the present experiments, the

structures in which the processes underlying resting activity and mechano-sensitivity of the ampullar receptors take place, cannot be positively identified The action of curare did reveal, however, that both processes are located in the hair-bearing pole of the sensory cells Kinocilium and stereocilia are therefore most likely to be the structures involved

ACKNOWLEDGEMENT

We are much indebted to Professor C Casella, Head of the Institute of General Physiology of Pavia, for his generous help and valuable suggestions in designing the experiments and in discussing the results

ZUSAMMENFASSUNG

Zur besseren Bestimmung des Effekts von D Tubocurarin bei Ampullarrezeptoren im Vestibularapparat des Frosches wurde die Substanz sowohl den isolierten semizirkularen Kanälen als auch den nichtisolierten Labyrinthen verabreicht Nur bei Anwendung in den isolierten semizirkularen Kanälen konnte das Curare je nach der angewandten Dosis (50–200 μg/ml) sowohl die Ruheaktivität der Ampullarrezeptoren als auch ihre Erwiderung auf die mechanische und elektrische Stimulierung vermindern oder beseitigen Sobald das Curare den nicht isolierten Praparaten verabreicht wurde, war hingegen fast kein Effekt feststellbar Die Aktion des Curare tritt bei jenen Rezeptoren, die für das Aktionspotential mit höheren Amplituden verantwortlich sind, fruher auf als bei den fur das mittlere und niedere Aktionspotential verantwortlichen Dieser Effekt kann als eine Erhohung er Schwelle ausgelegt werden, die sich auf die Gesamtit der die Crista Ampullaris bildenden Rezeptoren aushnt Die Curare Blockierung der mechanischen Erwierung in den Ampullarrezeptoren ist in etwa 15 Minuten nach Entzug der Substanz vollkommen reversibel, während sowohl die Ruheaktivität als auch die elektrische Erwiderung sich wesentlich rascher normalisieren und nach der Spulung sogar die Normalwerte übersteigen Die Lage und der mögliche Effektmechanismus des D-Tubocurarin in Ampullarrezeptoren wurden diskutiert

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MENIÈRE'S DISEASE

Preliminary Report of Lithium Treatment

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ract On the assumption that the hydrops of the lymphatic space present in Menière s disease is due defective transport of water and ions between the and perilymphatic space the authors in an open study treated patients with this disease with lithium onate as lithium among many biological effects acts transport of ions across membranes Our results ved that in 70% (21 patients) the effect of the treat t was excellent, judging by the patients' own state ts concerning the reduction in the frequency and rity of the attacks The remaining 30% (9 patients) ined no relief or only a doubtful effect of the treat t. Although a placebo effect cannot be ruled out his open trial the high (70%) and persisting (17 ths) effect does justify continued investigations into effect of lithium upon Meniere s disease

nough Menière's disease is clinically well ned, very little is known as yet about its logy and pathogenesis despite several in igations into the anatomical, physiological, biochemical changes

Nevertheless, studies on temporal bones of ents with Meniere's disease have disclosed arked dilatation of the endolymphatic space, cially the cochlear duct, saccule, and utricle uknecht, 1968) This so-called hydrops has n confirmed at operation (Portmann, 1973)

The hypotheses advanced concerning the se of this hydrops have mainly been centred two mechanisms Firstly, the vascular genesis ch tries to explain the changes by episodic ms or thromboses in the arterial blood supply of the inner ear (Lawrence, 1968) Secondly, the neurogenous theory, holding that changes in the tonus of the efferent nerve supply to the inner ear affects the ability of the labyrinthine membranes to maintain the high concentration of potassium in the endolymphatic space (Williams, 1965)

The pharmacological treatment of the disease has, therefore, been mainly channelled in two directions, one aimed at affecting the vascular system by vasodilating agents and the other towards the central and peripheral neurochemical mechanisms by means of sedatives, ganglion blockers, sympathicolytics, antihistaminics, etc —with varying success, according to the various sources (Altmann, 1955, Meyer, 1966)

A third hypothesis is that Meniere's disease is a local manifestation of a systemic disturbance in the regulation of the ratio between extracellular and intracellular fluid Therapeutic attempts have therefore been made with a diet low in fluid and salt as well as with diuretics, but with varying and doubtful results (Mygind & Dederding, 1938, Altmann, 1955)

However, it might be reasonable to look for a medication which could influence the transport of water and ions into and out of special biological compartments such as, for instance, the endolymphatic space Lithium might be a drug having these properties (Rafaelsen et al ,

1973, Singer & Rotenberg, 1973) Lithium is used in psychiatry for the treatment of manic-depressive psychosis Its effect upon mania is well documented (Schou et al, 1954), whereas that upon depression is still a moot point (Goodwin et al, 1969) The main indication is, however, its dependable and still unique prophylactic action both on manic and depressive states (Baastrup et al, 1970)

Lithium is administered in tablets containing 300 mg lithium carbonate The daily dose is 600–1 800 mg (16–48 milliequivalents) given in a single dose in the evening, aiming at a lithium concentration of 0 7–1 3 mmol/l in the serum drawn 12 hours after ingestion of the tablets

Correctly administered lithium medication gives but few unwanted effects (1) Tremor which responds to treatment with propranolol (Kirk et al, 1972), (2) polyuria with secondary polydipsia, (3) weight gain, and (4) goitre, which is usually euthyroid, but may be hypothyroid At serum lithium levels exceeding 1 3 mmol/l most patients develop incipient toxic symptoms in the form of nausea and vomiting, at times accompanied by diarrhoea, more marked tremor, as well as fatigue and drowsiness At even higher serum levels (above 2 0 mmol/l) these symptoms will be intensified and cerebral symptoms of various kinds will occur Severe poisoning may fatal Althouch the balance between therapeu and toxic serum concentrations of lithium is nely poised, experience has shown that lithium medication under careful supervision is a justified and safe treatment

Numerous investigations have been carried out with a view to elucidating the mechanism of lithium's effect in the hope that this would clarify the biochemical basis of manic-depressive psychosis (Rafaelsen & Mellerup, 1973, Schou, 1973) However, it must be admitted that the mode of action of lithium in this disease is still poorly understood

Lithium is predominantly used in psychiatry, but preliminary findings indicate that it may be of value also in thyrotoxicosis (Spaulding et al, 1972) and in Huntington s chorea (Dalen 1973 Mattson, 1973)

MATERIAL AND METHODS

Subjects

Forty out-patients with Meniere s disease investigated All patients fulfilled the f five subjective criteria (1) hearing imp attacks of (2) tinnitus, (3) vertigo, (4) n and (5) vomiting Objectively, all patie perceptive hearing loss and recruitmen sured by the method of Fowler and c Only patients with normal renal and c functions checked by normal serum cre and absence of proteinuria, normal ECG chest radiography, were included

The duration of the symptoms aver years, with a wide range—2 months to 20 y The frequency of the attacks was d thus 16 patients had daily attacks, 13 p about one attack weekly, and 11 patients one attack monthly

Treatment

All the patients had previously been tre an out patient basis by otological prac with antihistaminics, sedatives, and v tors, with little or no effect

At the commencement of treatme patients were informed about the effects of lithium and the signs of pois The initial dose was 600–900 mg lith bonate, maintenance dose 600–1 200 mg It was endeavoured to keep the serum concentration in the range 0 7–1.3 The concentration was checked week the first month, the next 6 months it was monthly, and thereafter every other Serum lithium was determined by flame metry (Amdisen 1967)

Therapeutic efficacy

The patients' own statements about the quency and severity of attacks were estimating the therapeutic efficacy

RESULTS

On the basis of the therapeutic effect the rial is divided into 3 groups (Table I)

Table I *Serum lithium concentrations, duration of treatment and of Meniere's disease in 40 patients*

...nt ...ories	No of patients	Average serum lithium concentration (range all values) (mmol/l)	Average duration of treatment (range) (months)	Average duration of disease (range) (years)
...I				
...under treatment with ...d effect	16	0 7 (0 3–0 9)	17 (3–30)	5 (1–15)
...ment discontinued ...atient, due to success of ...tment	5	0 7 (0 4–0 9)	14 (10–18)	
...II				
...ment discontinued ...to lacking effect	9	0 6 (0 3–0 8)	4 (1 12)	10 (1–20)
...III				
...ment discontinued ...to side effects	10	0 6 (0 4–1 0)	2 weeks (5–16 days)	5 (1–20)

...nsists of 21 patients in whom the thera...ic result was called excellent Sixteen of these ...nts were still on lithium, which they have ... taking for an average of 17 months (range ... 30 months) These patients have no symp... of their Meniere's disease, and only a few unwanted effects These effects were few, ...ively mild and have not affected the patients' ... to continue the treatment Another 5 ...nts interrupted the treatment of their own ...rd after an average of 14 months, as they perfectly well

...roup II is comprised of 9 patients who did ... respond properly to lithium therapy These ...nts took lithium for an average of 4 months ...ge 1–12 months)

...roup III is comprised of 10 patients in whom ...um was discontinued because of unwanted ...ts These effects occurred after medication ...n average of one week so that the patients ...ived lithium for about 2 weeks only

...he therapeutic effect became manifest in ...t patients after 2–4 weeks of treatment

...rom Table I it is apparent that the mean ...m level of lithium was 0 7 mmol/l (range of ...vels 0 3–1 0 mmol/l) With respect to serum ... the three groups did not differ from each ...r

No serum lithium level was above the therapeutic level, and indeed we have had no cases of toxic symptoms

As shown in Table I the average duration of the disease in group I was 5 years, and in group II 10 years This difference between the two groups was statistically significant (the Mann Whitney test, Siegel, 1956, $P<0\ 05$)

In Table II the frequency of the attacks shows, for group I, daily or weekly, 17, and monthly, 4 in group II, daily or weekly, 4, and monthly, 5 Using Fischer s exact probability test (Siegel, 1956) we found that group I had statistically significantly more often daily or weekly attacks ($P=0\ 06$)

The unwanted effects (Table III) were like those usually reported (Schou et al, 1971) but the severity was extremely varied Tremor of the hands was by far the most common effect, but the medication was never withdrawn for this reason as the tremor could be effectively controlled by propranolol 10 mg 3 times daily The tremor appeared after only a few days' medication Four patients out of 21 in Group I and one of the 9 in group II were treated with propranolol With the exception of insomnia, weight gain in one case, and fatigue in another, the other unwanted effects appeared soon

Table II *Distribution of frequency of attacks in 40 patients with Meniere's disease, treated with lithium*

Patient categories	No of patients with daily or weekly attacks	No of patients with monthly attacks	Total
Group I			
Good effect of lithium treatment	17	4	21
Group II			
Lacking effect of lithium treatment	4	5	9
Group III			
Patients with unwanted effects of lithium treatment	6	4	10

about one week of medication, and all gave occasion to discontinue the medication The effect of lithium upon Meniere's disease is illustrated by the following case history in which the patient continued the medication in spite of severe unwanted effects

Case History

A 52 year-old housewife referred to the University ENT Department, Rigshospitalet, by an ological practitioner with a diagnosis of Menre's disease There was no familial predisposition, especially not to manic-depressive psychosis.

Table III *Unwanted effects in 40 patients with Meniere's disease treated with lithium*

In order of frequency	No of patients
Tremor	10
Aggravation of symptoms	4
Fatigue	3
Nausea	3
Weight increase	2
Thirst and polyuria	1
Gastritis	1
Goitre and myxedema	1
Edemas	1
Precordial pressure	1
Insomnia	1

For 8 years she had been develop[illegible] creasing hearing loss in the left ear D[illegible] same period frequent attacks of sudden vertigo was accompanied by tinnitus and vomiting She had previously bee[illegible] with cinnarizinum (an antihistamine) any effect and with thiethylperazine (a thiazine) with some effect upon the but not upon the frequency of the atta[illegible] perceptive hearing loss was 65 dB and [illegible] pronounced recruitment measured by [illegible] and Fowler methods, and left sided canal

The patient was placed on lithium c[illegible] tablets, 900 mg/24 hours. The serum determinations were constant at 0.8 After about 3 weeks on this medica[illegible] patient obtained a marked improvem[illegible] that the attacks had stopped She had [illegible] effects of lithium in the form of tre[illegible] hands, fatigue, and nausea The tr[illegible] treated with propranolol 10 mg 3 ti[illegible] with a good effect Thereafter the c[illegible] was satisfactory and stable for 6-7 m[illegible] was then noted that she had had a w[illegible] of 15 kilograms The weight stabilized level, and after 10 months' treatment the wanted to go on taking lithium, de[illegible] marked weight gain, some tremor, and and as now she has only negligible complaints

DISCUSSION

As already mentioned the therapeutic was manifest after 2 to 4 weeks o[illegible] Therefore, the 10 patients in whom li[illegible] discontinued after only 2 weeks ha[illegible] excluded from the assessment This le[illegible] patients of whom 21 (70%) reported subjective result of the treatment, judged reduction in the frequency and se[illegible] attacks, whereas 9 patients (30%) had doubtful effect

The anamnesis of the remaining 30 [illegible] showed that the group with a good t[illegible] outcome had statistically significantly daily or weekly attacks and a statistic[illegible] nificant shorter duration of the disease

› *with a poor therapeutic outcome* Where- ne difference between the two groups in the uency of attacks is difficult for us to explain, finding that lithium is more apt to be ctive in patients with a short history agree the findings that the changes of the inner in Meniere's disease initially are reversible some years become permanent If our mption that lithium influences the transport ater and electrolytes into the endolymphatic e is correct, it must be expected that this can act only if some function of the mem- ie transport mechanisms remains, i e, in s with a short duration of the disease

e can only with reservations compare our minary results with other well substantiated ical treatments of Meniere's disease, be- e our follow up period is short

cNally (1953) demands a 5-year observa- period and preferably 5 years' freedom from ptoms, in assessing the results of medical tment of Meniere's disease Moreover, the ssment *should be based upon double-blind* s without or with cross over design As far e know, there have been only three double- d trials, viz, Arner et al's (1958), Philip- s (1962), and Collins & Poe's (1962) These ors have studied the effect of antihistaminics amphetamine upon Meniere's disease, and hree groups found a statistically significant t

ur lithium therapy was run as an open trial all its inborn risks of erroneous conclusions ough the good therapeutic result (70%) asses that usually found with placebo, and ough the effect has been maintained, for an age of 17 months, it cannot be denied that very patients have been good placebo onders

ith a view to the dosage and serum con- ation of lithium we have endeavoured to the patients within the range 0 7–1 3 mmol/l h empirically has been found necessary in treatment of manic-depressive psychoses ever, we do not know whether the same entrations are needed, as we have long been to keep one patient symptom free on a serum lithium concentration of only 0 3 mmol/l This patient, therefore, is not included in the analysis

The unwanted effects of the lithium therapy correspond to those reported from the lithium therapy of manic depressive psychosis (Schou et al, 1971) It is remarkable, however, that the patient who developed goitre showed this effect after only 2 weeks' medication We found a serum thyroxine level of 45 nmol/l (normal range 66–139 nmol/l), but there is no possibility of deciding whether the patient was hypothyroid prior to the treatment or whether the low serum thyroxine was induced by lithium

These preliminary findings may indicate that treatment with lithium has a favourable effect upon the symptoms in patients with Meniere's disease The results, moreover, justify further investigations, and in a joint project the University ENT Department and the Psychochemistry Institute, Rigshospitalet, have started a double-blind cross-over trial to confirm or disprove these *preliminary results*

ZUSAMMENFASSUNG

Es wird angenommen dass der bei der Menièrschen Krankheit charakteristische endolymphatische Hydrops auf einer abnormen Forderung von Wasser und Ionen zwischen den endolymphatischen und den perilymphatischen Raumen beruht Da Lithiumkarbonat unter vielen anderen biologischen Eigenschaften auch die Ionenwanderung durch Membranen beeinflusst, wurden Patienten mit der Menierschen Krankheit in einer offenen Pilot Study mit diesem Praparat behandelt *Ergebnis* 70% der Patienten (21) zeigten nach eigenen Beobachtungen ausgezeichnete Behandlungsergebnisse in bezug auf die Reduktion von Häufigkeit und Starke der Anfalle Bei den übrigen 30% (9) zeigte sich nur zweifelhafte oder gar keine Wirkung Obwohl in dieser offenen Behandlung eine Placebo-Wirkung nicht ausgeschlossen werden kann berechtigt der Anteil positiver Ergebnisse (70%) und zugleich deren Dauer (17 Monate) zu weiterer Untersuchung der Einwirkung von Lithium auf Patienten mit der Menierschen Krankheit.

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ÜBER DIE VISKOSITÄT DER LYMPHE IM INNENOHR DES HAUSSCHWEINES

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trakt Die Viskosität von Lymphe wird nach verschie-n Methoden, die insbesondere bezuglich der notdigen Korrekturfaktoren diskutiert werden, bestimmt Kapillarmessungen konnen nur Meßergebnisse ausertet werden, die oberhalb eines Grenzdruckes geinen werden Danach haben Peri und Endolymphe gleiche, etwa um 10% großere Viskosität als Wasser, och eine um den Faktor 1,6 kleinere Viskositat als tserum

1 EINLEITUNG

den letzten Jahren wurde damit begonnen, hydromechanischen Vorgänge im Innenohr ht nur für das System scala vestibuli–scala dia–scala tympani zu untersuchen, sondern h innerhalb der scala media (Helle, 1974, ele, 1973, Zwicker, 1971, 1972, 1974) Neu t hat bereits 1950 in Betracht gezogen, daß Erregung der Sinneszellen mit Strömungen der scala media zusammenhangen könnte ch v Illberg (1968) muß angenommen wer-ı, daß die Endolymphe nicht nur den sulcus ralis, sondern auch den Spalt zwischen Deckmbran und lamina reticularis erfullt Wenn r, wie an anderer Stelle diskutiert (Helle, 74 Zwicker, 1972, 1974) Strömungen in dien Spalt für die Verbiegung der Haare und mit fur die Erregung der Haarzellen maßgeb-ı waren, dann käme der Viskosität der Endonphe eine fur die Beschreibung der ablaunden Vorgange entscheidende Bedeutung zu

Verschiedene Angaben über die Viskosität der dolymphe und der Perilymphe sind von hnieder & Schindler (1964) gesammelt dargestellt und durch eigene Meßergebnisse ergänzt worden Letztere, sowie diejenigen von Money et al (1966) zeigen Viskositäten in der Nähe derjenigen von Wasser Die Angaben anderer Autoren (Schnieder & Schindler, 1964) weichen um Faktoren davon ab Es erscheint daher sinnvoll, die Ergebnisse nachzuprüfen und nach möglichen Gründen für Abweichungen zu suchen

2 MESSMETHODEN

a) Ostwald Viskosimeter

Bei ihrem Gebrauch wird eine bestimmte Flüssigkeitsmenge durch die von ihrem Eigengewicht hervorgerufene Kraft durch eine senkrecht stehende Kapillare definierter Länge hindurchgedrückt Sie sind im Handel erhältlich und sehr einfach in der Handhabung (Kohlrausch, 1968) Sie sind jedoch nur für Flüssigkeitsmengen von mindestens etwa cm³ verwendbar Die Peri- und Endolymphmengen, die einem Innenohr entnommen werden können, liegen jedoch in der Größenordnung mm³, so daß das Ostwald–Viskosimeter für die Messung der Viskosität von Lymphe nicht in Frage kommt Für Vergleichsmessungen an Eichflüssigkeiten ist es wegen seiner großen Genauigkeit sehr geeignet und wurde dazu auch benutzt

b) Kapillarviskosimeter

Sie bestehen aus horizontal liegenden Kapillaren mit kleinem Innendurchmesser $\varnothing_K$, in denen

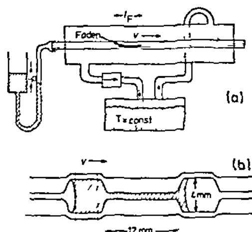


Abb 1 (*a*) Schematischer Aufbau zur Messung der Viskosität mit Hilfe der Verschiebungsgeschwindigkeit v eines Flüssigkeitsfadens der Länge l_F in einer Kapillare infolge der Druckdifferenz Δp (*b*) An zwei Stellen zu Zylindern aufgeweitete Kapillare Bei diesem als „Miniviskosimeter" bezeichneten Aufbau wird die Flussigkeitsmenge (etwa 20 mm³) durch Δp an den beiden Marken (gestrichelt) vorbeigeschoben und v bestimmt

durch äußere Druckdifferenz Δp ein Flussigkeitsfaden der Länge l_F verschoben wird Dem Hagenpoiseuille'schen Gesetz entsprechend gilt (Kohlrausch, 1968) folgender Zusammenhang

$$\Delta p = \frac{32\eta \cdot l_F \quad v}{\varnothing_K^2} \qquad (1)$$

aus dem sich die Viskosität η mit der Verschiebungsgeschwindigkeit v des Flüssigkeitsfadens rrechnen läßt Für eine Flussigkeitsmenge von m³ ergibt sich bei $\varnothing_K = 0{,}35$ mm eine Fadenge von etwa 1 cm Weil während der Verschiebung des Flussigkeitsfadens an seinem Ende immer etwas Flussigkeit an den Kapillarinnenwänden zuruckbleibt, ändert sich die Fadenlänge während der Messung ein wenig Damit dieser Einfluß gering bleibt, sollten Fadenlängen unter 1 cm nicht benutzt werden Da die Viskosität stark von der Temperatur abhängig ist, wurde die Kapillare (Abb 1*a*) in eine weitere Glasröhre so eingebaut, daß sie von Wasser konstanter Temperatur umspült werden konnte Der Körpertemperatur des Hausschweines entsprechend wurden alle Untersuchungen bei 39 C durchgeführt Die Druckdifferenz Δp zwischen den Enden der 40 cm langen Kapillare wurde durch ein kommunizierendes Röhren-Schlauchsystem eingestellt und in cm W. säule (Ws) abgelesen

Bei Flüssigkeitsfadenlängen, die nicht ganze Kapillarenlänge erreichen, ist die D differenz, die zwischen den Kapillar herrscht, nicht gleich der Druckdifferenz zwischen den beiden Enden des Flüssig fadens wirkt, weil ein Teil der ersteren über Luftsäule in der Kapillare abfällt, die verschoben werden muß Demnach m Gesamtdruckdifferenz Δp aufgeteilt wer die Druckanteile Δp_L und Δp_s, die zur schiebung der Luftsäule bzw des Flüss fadens notwendig sind

Dabei gilt $\Delta p = \Delta p_L + \Delta p_s$

Für die Auswertung wichtig ist led Druckdifferenz Δp_s, die über einen Kor faktor k aus Δp bestimmt werden kan (1) und (2) ergibt sich

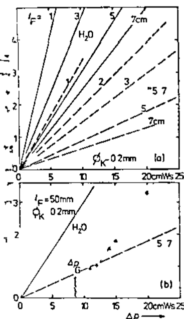
$$k = \frac{\Delta p_s}{\Delta p} = 1 - \frac{1}{1 + \frac{\eta_s}{\eta_L}\frac{l_{Fs}}{l_{FL}}}$$

Allerdings muß die Viskosität η_s der Fl etwa bekannt sein, um die Korrektur d führen zu können In Tabelle I sind die K turfaktoren k für eine Kapillarenges von 40 cm sowie fur das benützte „Mini simeter" (siehe unten) angegeben

Eine Abschätzung der Viskositäten k durch vorgenommen werden, daß ans $v = f(\Delta p)$ die für eine Verschiebung des F

Tabelle I *Korrekturfaktoren k_{H_2O} und k Wasser und Eichflüssigkeit für versch Flüssigkeitsfadenlängen bei 40 cm Ges larenlänge und für das „Miniviskosimeter"*

	L_F [cm]	k_{H_2O}	k
Kapillare 40 cm lang	1	0.47	[illegible]
	3	0.74	[illegible]
	5	0.83	[illegible]
Miniviskosimeter		0.85	[illegible]



…b 2 Verschiebungsgeschwindigkeit *v* als Funktion von … bei einem Kapillardurchmesser $Ø_K = 0\ 2$ mm (*a*) für Flüssigkeiten H_2O (durchgezogen) und 5 7 (gestrichelt) mit der Flüssigkeitsfadenlänge l_F als Parameter (*b*) Endolymphe (Δ) und Perilymphe ( ) im Vergleich mit den Ergebnissen aus (*a*) für $l_F = 5$ cm Δp_G …echnet den bei Lymphe auftretenden Grenzdruck

…itsfadens um Δl (in unserem Fall $\Delta l = 20$ cm) notwendige Zeit als Funktion der Fadenlänge …mit Δp als Parameter aufgetragen wird Alle …urven müssen Geraden sein, die sich auf der negativen Achse bei l^*_{Fx} in einem einzigen Punkt schneiden Aus (1) und (2) ergibt sich mit $v = \Delta l / t$ und einer Konstanten A

$$t = A\ \frac{\eta_L\ l_{FL} + \eta_x\ l_{Fx}}{\Delta p} \qquad (4)$$

Wenn $l_{Fx} < l_{FL}$ kann l_{FL} als konstant und die Gleichung

$$t = \frac{A\ \eta_x}{\Delta p}\ l_{Fx} + \frac{A\ \eta_L\ l_{FL}}{\Delta p} \qquad (5)$$

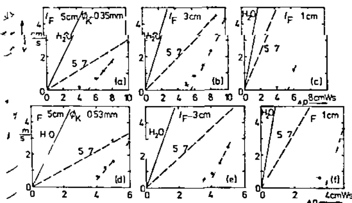
als eine Schar von Geraden mit dem Parameter Δp angesehen werden, die sich im Punkt

$$l^*_{Fx} = -\frac{\eta_L}{\eta_x} l_{FL} \qquad (6)$$

schneiden (Für die mit $Ø_K = 0\ 35$ mm gewonnenen Ergebnisse (Abb 3, oben) ist die entsprechende Umzeichnung in Abb 4 für die Flüssigkeiten H_2O und 5 7 durchgeführt) Die Viskosität von Luft hat bei 39°C den Wert $\eta_L = 0\ 019$ cp Damit läßt sich aus der umgeschriebenen Gl (6) η_x abschätzen

$$\eta_x = -\frac{l_{FL}}{l^*_{Fx}} l_{FL} \qquad (7)$$

Für die Bestimmung des Korrekturfaktors ist diese Abschätzung die meist eine Genauigkeit von 20 % besitzt ausreichend Vorteilhaft ist ferner, daß Fehlmessungen, z B durch Verun



…b 3 Wie Abb 2 (*b*) jedoch mit $Ø_K = 0\ 35$ mm und …n Parametern $l_F = 5$ cm (*a*) $l_F = 3$ cm (*b*) l_F 1 cm (*c*) … $Ø_K = 0\ 53$ mm und den Parametern $l_F = 5$ cm (*d*) $l_F = 3$ cm (*e*) $l_F = 1$ cm (*f*) Außer für Perilymphe (+) sind auch Meßwerte für Blutserum (○) eingetragen.

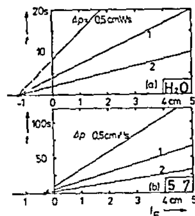


Abb 4 Für eine Verschiebung des Flussigkeitsfadens um 20 cm notwendige Durchlaufzeit *t* als Funktion der Fadenlänge l_F für die Flüssigkeiten H_2O (*a*) bzw 5 7 (*b*) Parameter ist die Druckdifferenz Δp (Methode 2*b*)

reinigungen hervorgerufen, in Darstellungen entsprechend Fig 4 sofort erkannt und eliminiert werden können

c) *„Miniviskosimeter"*

Diese Art von Viskosimeter ist eine Kombination der Methoden *a* und *b* Es benotigt eine Flüssigkeitsmenge von nur etwa 20 mm³ Eine Kapillare mit einem Durchmesser von 0,53 mm wurde an zwei Stellen im Abstand von 20 mm so aufgeblasen, daß zwei etwa zylindrisch geormte Behälter mit einem Innendurchmesser 4 mm entstanden (Abb 1*b*) Nachdem die igkeit eingefullt ist, wird sie wie bei Meóde 2*b* durch die Druckdifferenz Δp in Bewegung gesetzt Die Verschiebungsgeschwindigkeit wird aus der Zeitdauer *t* bestimmt, welche die Flüssigkeit benötigt, um mit ihrem linken Rand von der einen zur anderen Markierung (gestrichelt in Abb 1*b* eingezeichnet) zu gelangen Da die Druckdifferenz auf verhältnismäßig großen Flächen angreift und demnach wesentlich größere Krafte erzeugt als bei Methode 2*b*, stören kleine Druckänderungen, die im Röhren-Schlauch-System bei Temperaturschwankungen entstehen Deswegen wurde vor jeder Druckeinstellung der 0-Punkt (Δp 0) durch Öffnung eines Abzweighahnes kontrolliert

3 DIE ENTNAHME DER LYMPHE

Die Felsenbeine frisch geschlachteter [illegible] schweine wurden im Schlachthof aus[illegible] (Zwicker, 1971) Zur Entnahme von Endolymphe wurde unter dem Operationsmikroskop die [illegible] nenohr nahe dem Helicotrema eröffnet ([illegible] vestibuli) und zunächst etwas Perilymphe [illegible] nommen, so daß der Flüssigkeitsspiegel die Reiss[illegible] nermembranen freigab Mit einer feinen P[illegible] wurde danach die Reissnermembran d[illegible] stoßen und ein Teil der Endolymphe abge[illegible] In einigen Kontrollexperimenten wurde eventueller Durchbruch der Reissnerme[illegible] an anderer Stelle dadurch kontrolliert, da[illegible] der Entnahme von Endolymphe die Peri[illegible] im Durchspulverfahren durch eine ange[illegible] Flüssigkeit ersetzt wurde War die Endol[illegible] ohne Anfärbung, so war die Reissnerme[illegible] nicht gebrochen und die Endolymphe nicht [illegible] Perilymphe vermischt Durchbruche tra[illegible] auf, wenn zuviel Endolymphe abgesaugt [illegible]

Perilymphe wurde entweder durch das [illegible] Fenster oder/und am Helicotrema entno[illegible] Sie war einfacher und auch in größerer [illegible] zu gewinnen als Endolymphe

Die Präparation der Lymphflüssigkei[illegible] meist nach einer Stunde post mortem be[illegible] Wenn nicht anders angegeben, wurden die [illegible] sungen innerhalb der zweiten Stunde dur[illegible] führt, die Lymphe demnach nicht länger [illegible] Stunden post mortem benutzt Fast jede [illegible] reihe wurde mit Lymphe von einem [illegible] Tier durchgefuhrt Weil sich dabei keine [illegible] fikanten Unterschiede ergaben, wurde Ly[illegible] auch aus verschiedenen Ohren gesammelt[illegible] daß mit größeren Fädenlängen (Metho[illegible] und mit dem „Miniviskosimeter" g[illegible] werden konnte

4 ERGEBNISSE

Neben Endolymphe und Perilymphe [illegible] zum Zwecke des Vergleichens und des E[illegible] reines Wasser und eine Eichflüssigkeit be[illegible] Als Eichflüssigkeit diente eine Mischu[illegible]

belle II *Die nach den beschriebenen Methoden gewonnenen Viskositäten Temperaturen und* *-hten sind ebenfalls angegeben*

			Viskositat			
-issigkeit	Temp [°C]	rel Dichte	Nach 2*a* abs [cp]	rel	Mittelwerte nach 2*b* rel	Nach 2*c* rel
isser	20		1 002			
	39	1	0 659	1	1	1
-itserum (Hausschwein)	39	1,035	1,13	1,73	1,72	1 69
7	39	1 37	2,52	3 82	3 80	3 70
-mphe ungefiltert	39				1,17	1,20
-mphe gefiltert	39					1 09

-iromschwefelsäure[1] und dest Wasser (H_2O) Volumverhältnis 5 7, die im Folgenden als 7' bezeichnet ist Nachdem sich eigenartige -bhängigkeiten der Verschiebungsgeschwindig -nten vom Druck sowohl fur die Peri- als auch r Endolymphe ergaben (*siehe unten*), wurde -tutserum (Hausschwein, 2 mal 20 min bei 00 Umdr/min zentrifugiert) mit in die Meß--ihen einbezogen, weil es nicht nur ähnliche -fekte zeigte, sondern auch in großerer Menge -trahiert werden konnte

a) Mit der unter 2*a* beschriebenen Methode -urden die Viskositäten aus der Durchlaufzeit stimmt Es ergaben sich die in Tabelle II -gegebenen Werte, bei denen die fur die Be -chnung notwendige Konstante aus den fur asser bei 20°C gemessenen Durchlaufzeiten it Hilfe der bekannten Viskositat $\eta_{H_2O\ 20\ C}$ 1 002 cP bestimmt wurde (mittlerer rel Feh- etwa 1 %)

Die fur das Blutserum des Hausschweines ge- ndenen Werte liegen innerhalb des fur Human- rum von Rauch (1964) angegebenen Viskosi- tsbereiches

b) Die nach Methode 2*b* gemessene Verschie -ngsgeschwindigkeit *v* des Flussigfadens ist in bb 2*a* als Funktion der Druckdifferenz Δp rgestellt Sowohl fur die Chromschwefelsäure- assermischung (5 7, gestrichelt) als auch für $_2$O (durchgezogen) ergeben sich Ursprungs raden Fur Endo- und Perilymphe dagegen dies nicht der Fall In Abb 2*b* sind Meß- erte dafur im Vergleich mit H_2O und 5 7 angegeben Bei kleinen Drucken bis 5 cm Ws bewegt sich der Flussigkeitsfaden aus Lymphe gar nicht Bei Drucken bis 10 cm Ws bleibt der Faden häufig hängen Erst oberhalb $\Delta p = 10$ cm Ws kann einigermaßen sicher gemessen werden *Die Geschwindigkeit v steigt dann mit wachsendem Druck etwa so an, wie für H_2O* Die Neigung der Geraden ist für Lymphe nur wenig geringer als für H_2O Endolymphe (Δ) und Perilymphe (+) verhalten sich innerhalb der Meßgenauigkeit gleich Dies wurde auch bei anderen Kapillardurchmessern bestatigt, so daß die Viskositäten von Endo- und Perilymphe als gleich angesehen werden konnen

In Abb 3 sind fur $\varnothing_K = 0{,}35$ mm (obere 3 Diagramme) bzw fur $\varnothing_K - 0{,}53$ mm (untere 3 *Diagramme) weitere solche Abhängigkeiten fur* Flüssigkeitsfadenlangen $l_F = 50$ mm (links), $l_F -$ 30 mm (Mitte) und $l_F = 10$ mm (rechts) dargestellt Neben Perilymphe, H_2O und 5 7 wurde auch Blutserum ausgemessen Perilymphe und Blutserum verhalten sich auch unter den gean derten Bedingungen ähnlich wie in Abb 2*b* Ist ein Grenzdruck Δp_G (Schnittpunkt der punktiert eingetragenen Asymptote mit der Δp-Achse) überschritten, zeigt die Abhangigkeit (punktierte Gerade) für die Perilymphe bei allen Meßbedingungen eine etwas geringere Neigung als diejenige fur Wasser Fur Blutserum verlaufen die Kurven (strichpunktiert) noch flacher Dies bedeutet, daß Perilymphe eine etwas größere Viskosität als Wasser besitzt und Blutserum noch eine größere, wenn von dem Effekt des Grenzdruckes zunächst einmal abgesehen wird

Die nach der Methode 2*b* bestimmten Visko-

[1] Chromschwefelsäure ist eine Mischung aus H_2SO_4 onzentriert) und 2 % $K_2Cr_2O_7$ (wasserfrei)

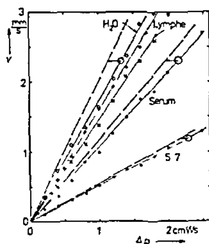


Abb 5 Die am „Miniviskosimeter" gemessene Verschiebungsgeschwindigkeit v als Funktion der Druckdifferenz Δp für die Flüssigkeiten 5 7, Blutserum, Lymphe ungefiltert (×) Lymphe gefiltert (+) und H_2O (O) Für 5 7, Serum und H_2O sind die korrigierten gegen den Uhrzeigersinn verdrehten Mittelwertsursprungsgeraden gestrichelt eingetragen

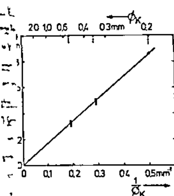
sitätswerte relativ zu Wasser bei 39°C sind in Tabelle II eingetragen Sie sind Mittelwerte aus allen nach dieser Methode gewonnenen Ergebnissen, wobei für Lymphe und Blutserum die Steigungen der Asymptoten (Abb 3 punktiert bzw strichpunktiert) der Bestimmung zugrunde gelegt wurden

c) Das „Miniviskosimeter" besitzt nicht nur inen bei der Messung von Flüssigkeit erfüllten l der Kapillare, sondern links und rechts von ı Zylindern (Abb 1*b*) noch zwei weitere, von uft durchströmte Kapillarenteile Letztere besitzen eine Länge von insgesamt 70 mm gegenüber 12 mm von Flüssigkeit erfüllter Kapillarlänge, so daß auch hier, wie unter 2*b* beschrieben, eine Korrektur durchgeführt werden muß In Tabelle I sind Werte für den Korrekturfaktor k angegeben Abb 5 zeigt die gemessenen Verschiebungsgeschwindigkeiten v in den Zylindern als Funktion des Druckes Δp In der Kapillare strömt die Flüssigkeit etwa 60mal schneller Die durchgezogenen Geraden stellen Ursprungsgeraden dar, welche die Meßpunkte am besten annähern Bei der Gewinnung von 20 mm³ Lymphe wurde auf eine Trennung von Endolymphe und Perilymphe verzichtet Dies schien erlaubt, nachdem sich bei Messungen mit einem Gemisch der Lymphen mit Methode 2 Unterschiede gegenüber reiner Peri- bzw lymphe ergeben hatte Die Messung an filterter Lymphe konnte innerhalb von den, die mit gefilterter erst innerhalb Stunden post mortem abgeschlossen Für die Flüssigkeiten H_2O, Serum und 5 die korrigierten Ursprungsgeraden, a die Viskosität bestimmt wurde, gestrichelt getragen Die relativen Viskositäten, die Art bestimmt wurden, sind in Tabelle I geben

5 DISKUSSION

Endolymphe und Perilymphe zeigen gleich halten bei Kapillarviskositätsmessungen schiede gehen in der Meßgenauigkeit u fällig ist das Auftreten eines Grenzdruck bei dieser Methode für Lymphe und für unterhalb dessen sich der Flüssigkeitsfaden bzw stark stockend bewegt Demnach s Kositätsbestimmungen nach dieser Meth einer einzigen Messung bei einem best Druck Δp nicht möglich Eine Ursprun durch diesen Punkt wurde zu falschen zu großen Viskositätswerten führen Wi gegen der Druck über den Grenzdruck g und die Asymptotenneigung als Grundl Viskositätsbestimmung herangezogen geben sich Werte, die in guter Übereins mit solchen Werten liegen, die mit Methoden gewonnen wurden

Das Auftreten eines Grenzdruckes ist d fälligste Abweichung der Druckdiffer schwindigkeitskurven von Lymphe und Se genüber denjenigen der Eichflüssigkei Größe hängt nicht von der Flüssigkeits länge ab (Abb 3), wohl aber vom Ka durchmesser (Abb 2*b* und Abb 3) W Viskosität von Lymphe und Serum jenigen von Bingham'schen Flüssigke gleichbar, so müßte der Grenzdruck Fadenlänge anwachsen Daß er dies n weist darauf hin, daß nur die beiden Fa nicht aber der Faden selbst mit der E des Grenzdruckes in Zusammenhang werden können An den Fadenenden



6 Grenzdruck Δp_G (aus Fig 2b und Fig 3 entnom-
) als Funktion der Kehrwertes des Kapillardurch
ers $Ø_K$ Die Große von $Ø_K$ kann der oberen Skale
ommen werden

dunne Häutchen an der Oberflache bilden hangen an der Wand fest und müssen bei schiebung des Flüssigkeitsfadens abgerissen durchbrochen werden Sie bilden sich aber der, sobald die Oberfläche zur Ruhe kommt, der Flussigkeitsfaden still steht Dies ist ächst eine Annahme Aus ihr kann jedoch Abhangigkeit des Grenzdruckes vom Ka- ardurchmesser vorhergesagt werden, wenn enommen wird, daß die Dicke der sich bil- den Hautchen vom Kapillardurchmesser un- angig ist Das Fadenende hat näherungs- se die Form einer Halbkugel Für Hohl eln, bei denen die Wandstärke, d h die utchendicke h sehr klein ist gegenüber dem dius r der Kugel ist der Überdruck

$$\ \ = \frac{2h}{r}\,\delta_t \qquad (8)$$

bei δ_t die auftretende tangentiale Spannung Hautchen ist Wird ihr Grenzwert δ_{tG} (beim reißen) ebenso wie ihre Dicke h als konstant enommen, so wird

$$\frac{4h}{Ø_K}\,\delta_{tG} \sim \frac{1}{Ø_K} \qquad (9)$$

bei als Durchmesser der Halbkugel der Durch- sser der Kapillare eingesetzt ist Der Grenz- ck mußte also mit wachsendem Kapillar durchmesser abnehmen In Abb 6 ist der den Abb 2b und 3 entnommene Grenzdruck als Funktion des Kehrwertes des Kapillardurch- messers aufgetragen Die Meßwerte liegen er- staunlich genau auf einer Ursprungsgeraden, wie sie Gleichung (9) vorschreibt, so daß die An- nahme der sich bildenden Häutchen als bestä tigt angesehen werden kann Bei großen Ober- flächen, d h großen Durchmessern wird der Grenzdruck sehr klein, so daß er beim „Mini- viskosimeter" praktisch in der Meßgenauigkeit untergeht

Die Vorstellung über das Entstehen des Grenz- druckes konnte dadurch bekräftigt werden, daß es gelang, Flüssigkeitsfaden aus Blutserum auch bei Drucken weit unter dem Grenzdruck zu ver- schieben, wenn beiden Enden je ein kurzer Flüs- sigkeitsfaden aus Ol angelagert wurde Aller- dings beginnt sich schon nach wenigen Ver- schiebungen eine Emulsion zu bilden, was sofort zum ursprünglichen Verhalten fuhrt, so daß keine systematischen Messungen durchgefuhrt werden konnten Die Lymphflussigkeit selbst verhält sich demnach wie eine ideale Flussigkeit, mindestens im untersuchten Scheergefällebereich Nach Klärung der insbesondere bei Kapillar viskosimetern auftretenden, stark störenden Nebeneffekte kann zusammenfassend folgendes festgestellt werden

In Übereinstimmung mit den Angaben von Schnieder & Schindler (1964) bzw von Rauch (1964) werden Viskositaten fur Endo- und Peri- lymphe gefunden, die nur etwa 10% uber der von Wasser liegen, jedoch deutlich (Faktor 1,6) unter derjenigen von Blutserum

Fraulein Dipl Biologe A Frei hat bei den Untersuchun- gen mit dem „Miniviskosimeter", Herr Dipl Ing. G Appel bei den Untersuchungen mit der Kapillarmethode tatkräftig mitgeholfen Beiden mochte ich sehr herzlich danken ebenso Herrn Veterinardirektor Dr Schneida wind und seinen Mitarbeitern von der Sanitätsanstalt des Munchner Schlachthofs fur ihr verstandnisvolles Entgegenkommen Herrn Dipl Ing R Helle und Herrn Dr Ing E Terhardt bin ich fur wertvolle Diskussionen dankbar

Die Arbeit entstand im Sonderforschungsbereich „Kybernetik" Munchen, der von der Deutschen For schungsgemeinschaft unterstutzt wird.

SUMMARY

The viscosity of lymph is determined by using different methods which are discussed especially in regard to the necessary correction coefficient When using capillaries to measure viscosity, only such data can be taken into account as are produced by a pressure larger than critical The viscosity of endolymph and of perilymph is found to be equal and 10% larger than the viscosity of water, respectively, yet a factor of 1 6 smaller than the viscosity of serum

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PERSTIMULATORY SUPRATHRESHOLD ADAPTATION

III Sensorineural Deafness

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(Received December 12, 1973)

tract Perstimulatory suprathreshold adaptation was lied in 243 patients with various types of sensorineural ring loss. The technique was based on the continuous aural loudness balance the comparison tone consisting pulses of 200 msec and intervals of the same length asurements were carried out at 20 40 or 60 dB SL frequencies 1 000–4 000 Hz. In primary hair cell ons adaptation was slighter than in normally hearing and it tended to decrease with increasing threshold Very pronounced adaptation values were found in ocochlear deafness In central nervous lesions they e normal

e of the first controlled studies of perstimula-y suprathreshold adaptation was made by od (1950) His series included 25 unilateral es of Meniere's disease A continuous tone of dB SL (sensation level) was presented at 00 Hz to both ears and the patient instructed balance the tone in the normal ear so as to ıal in loudness the one in the diseased ear e balance tracing showed in 20 cases a decrease 10 to 50 dB in 3 minutes, indicating increased ıptation in the impaired ear Palva (1955) d continuous tone balance periods of 15 sec ıng stimulation of the impaired ear with ılar tones for 3 min, and observed large ıptation (30 dB or more) in 14% of 51 ruiting perceptively deafened ears In the jority of cases adaptation was slight These asurements were carried out at 60 and 80 dB ›ve normal threshold for frequencies 500 to 00 Hz

s work was aided by a grant from the National earch Council for Medical Sciences

In Pestalozza's modification the loudness level loss was determined with a balance made during 5–10 sec after stimulation of 3 min at 50 dB SL (Cioce & Pestalozza, 1960, Pestalozza & Cioce, 1962), the series included 57 perceptively impaired ears In normal ears loudness loss of 15 dB was reported for frequencies 1 000–4 000 Hz In 18 cases of pure end organ lesions adaptation was generally within normal limits or slightly increased in early stages In VIII nerve lesions (5 cases) the test gave abnormally large values regardless of the degree of hearing loss In patients with central nervous lesions loudness level loss was within normal limits and abnormal adaptation was reported in only two cases with possible lesions of subcortical structures

Other reports include those of Pestalozza (1953) and Bosatra (1957), who presented examples of Meniere's disease and toxic end organ lesions associated with large adaptation values In presbyacusis Manzini et al (1956), using Pestalozza's technique on 44 patients, reported that half of the cases showed adaptation within normal values (10–15 dB) The remaining cases showed either pathological adaptation, i e loudness level loss of 25–30 dB (22 7% of the patients), or only slightly elevated values In 18 cases of skull injury Cioce & Spelta (1963) found increased adaptation in presumably central lesions whereas in cases with peripheral lesion the results showed normal or slightly increased values

Table I *Distribution of the material into clinical groups*

Diagnosis	No of cases
Cochlear lesions	
Noise injury	77
Meniere s disease	29
Streptomycin damage	4
Retrocochlear lesions	
Acoustic neurinoma	8
Cerebellopontine arachnoid cyst	1
Central lesions	16
Functional hearing loss	2
Mixed group	
Presbyacusis	25
Angiosclerotic degeneration	26
Heredodegenerative deafness	14
Skull injury	14
Non defined sensorineural deafness	27
Total	243

MATERIAL AND METHODS

The material consisted of 243 adult patients with various types of sensorineural hearing loss (Table I)

In all subjects air and bone conduction thresholds were determined by the usual descending-ascending method using Madsen Model OB 60 audiometer calibrated to ISO 1964 readings … reception thresholds and speech discrimi…on were measured as described by Palva (…52) Loudness recruitment was studied by the Fowler or Reger method, or in cases with insufficient threshold differences, taking the amplitude size of the threshold tracings as criterion by a self recording audiometer (Palva, 1957) Threshold tone decay for continuous tones was measured by the selfrecording audiometer during 3 min for the same frequencies as the suprathreshold adaptation

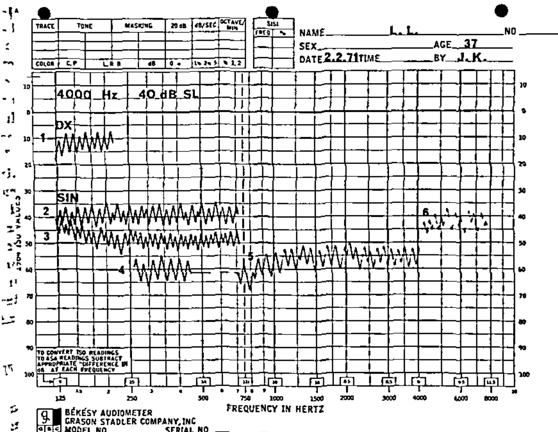
The apparatus and the testing procedure for measuring suprathreshold adaptation were identical with those described earlier (Kärja, 1968 and 1970) The measurements were carried out at 40 or 60 dB SL—in a few cases at 20 dB level—at suitable frequencies between 1 000 and 4 000 Hz so that the loudness level did not exceed 95 dB Determination of air conduction thresholds with interrupted signal by … Stadler Audiometer Model E 800 for … quency studied was followed by prestim… balancing (Fig 1) An interrupted pu… stimulus was presented to the test ear a… sensation level while a similar compari… with pulses and intervals of 200 mse…

i e continuous, stimulus was introduced … experimental ear for 3 min Balancing … interrupted tone was continued throug… stimulation Recovery of the threshold … test ear was recorded for 60 sec after c… of stimulation Adaptation in dB w… from the difference between adaptatio… and the prestimulatory balance level

RESULTS

Cochlear lesions

This group included 77 patients wi… injury, 29 with Menière's disease and … streptomycin damage Ages varied fro… 57 years and averaged 33 2 years All … complete loudness recruitment Speech discrimination rate was in mos… cases between 70 and 90 %, while 4 patie… Menière's disease and 3 with severe noi… had scores lower than 50 % Thresho… decay in 3 min was less than 30 dB … four ears with noise injury and in tw… Menière's disease, in which it was betw… and 47 dB The results for persist… suprathreshold adaptation in cochlear … are shown in Table II The values wer… ($p<0\,05$) than those measured in the … material (Kärjä, 1968) and in conducti… ness (Karja, 1970) The individual values … cases of streptomycin damage and … injury are seen in Fig 2 There was a t… for adaptation values to decrease with in… threshold loss This was not so in patie… Meniere's disease (Fig 3) The individual … tion values in all categories showe… variability Slowly developing adaptatio…



Perstimulatory suprathreshold adaptation measured at 4 000 Hz 40 dB SL on the left ear of a patient with noise injury Top left is shown the threshold tracing of the right ear (1) The tracings for the left ear are registered both with interrupted (2) and continuous test tone (3) for 3 min Bottom left is seen the prestimulatory balancing (4) In the middle the adaptation curve (5) shows loudness level loss of 4 dB in 3 min The recovery of the post-stimulatory threshold for the left (test) ear recorded with the interrupted test tone is shown on the right (6)

e II *Cochlear deafness Perstimulatory suprathreshold adaptation (dB) in 3 min prestimulatory ice level (dB) and post-stimulatory threshold shift (dB) 1 min after stimulation Stimulus intensity nd 60 dB above threshold level*

	Stimulus variables											
	1 000				2 000				4 000			
	40		60		40		60		40		60	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
ear deafness												
tation	11 0	7 3	12 8	11.3	15 8	10 0	15 1	13 5	12 1	10 8	8 1	8 4
mulatory balance	48 2	6 7	62 7	9 8	52 7	8 0	63 5	14 2	42 5	14 1	64 9	9 9
stimulatory shold shift	6 6	4 8	5 9	5 0	3 8	2 2	6 3	4 3	5 6	4 7	5 1	3 6
al ears (Kärjä,)	18 2	13 3	22 6	16 2	21 7	12 3	28 3	17 9	20 5	11 2	28 4	15 0
tation	41 0	8 3	58 9	10 0	40 6	8 4	57 4	11 4	37 9	8 1	57 1	8 2
stimulatory shold shift	3 2	2 7	4 0	2 6	3 6	3 4	4 0	3 2	3 6	3 2	3.3	2.4

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The apparatus and the testing procedure for measuring suprathreshold adaptation were identical with those described earlier (Kärja, 1968 and 1970) The measurements were carried out at 40 or 60 dB SL—in a few cases at 20 dB level—at suitable frequencies between 1 000 and 4 000 Hz so that the loudness level did not exceed 95 dB Determination of air conduction thresholds with interrupted signal by Stadler Audiometer Model E 800 for quency studied was followed by prestin balancing (Fig 1) An interrupted pu stimulus was presented to the test ear at sensation level while a similar compans with pulses and intervals of 200 msec was fed to the contralateral ear The pul simultaneous in both ears When the sub recorded the balance for 30 sec an i e continuous, stimulus was introduced experimental ear for 3 min Balancing interrupted tone was continued through stimulation Recovery of the threshold test ear was recorded for 60 sec of stimulation Adaptation in dB was ca from the difference between adaptatio and the prestimulatory balance level

RESULTS

Cochlear lesions

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e III *Cochlear deafness Amplitudes (dB) of pre- and post stimulatory thresholds, and excursion* *ıs of prestimulatory balance and adaptation tracings*

	Stimulus variables											
	1 000				2 000				4 000			
	40		60		40		60		40		60	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
ear deafness												
m threshold	8 2	2.9	7 5	2.5	6 9	1 4	7 1	2.0	6 8	2.0	6 7	3 0
m balance	9 8	2 8	9 1	3 0	7 3	2 6	8 6	4 4	7 4	2 6	9 1	3.2
tation tracing	12 4	5 0	9 8	3 5	8 8	3 8	9 9	4 8	8 8	3 6	7 7	2 6
stim threshold	8 6	3 2	7 2	2.1	7 4	2 7	7 0	2 6	7 3	2 7	7 1	3 1
al ears (Karja,)												
m threshold	6 0	1 8	5 6	1 9	5 6	1 8	5 2	1 7	5 6	1 7	5 0	1 2
m balance	7 1	2 9	6 5	2.2	7 3	2.8	6 6	2 3	7 0	2 5	6 6	3 1
tation tracing	7 0	2 6	7 1	2.6	7 1	3 2	7 0	2 6	6 8	2 4	6 8	2 8
tim threshold	5 8	1 8	5 8	2 0	6 8	1 8	5 4	2.0	5 4	1 6	5 4	1 9

others suffered from localized vascular disances

reshold tone decay (2 000–4 000 Hz) was han 30 dB in all the ears studied Loudness ce was incomplete in 7 patients and negan the others The values for perstimulatory athreshold adaptation (Fig 5) were equal to normal as were also the prestimulatory balance levels and post-stimulatory threshold shifts and the excursion widths of the tracings

In addition, 2 patients with functional hearing loss were studied The measurement of suprathreshold adaptation was successful in both even though there was some uncertainty as regards

e IV *Mixed group of sensorineural deafnes Perstimulatory suprathreshold adaptation, prestimu-* *) balance and post stimulatory threshold shift*

xplanations see Table II

	Stimulus variables											
	1 000				2 000				4 000			
	40		60		40		60		40		60	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
with complete *ıment*												
tation	14 6	5 7	7 3	7 8	14 6	10 4	10 9	12.4	8 4	9 0	13 4	11 9
mulatory balance	52 3	6 9	66 6	9 4	56 6	16 8	62.4	10 5	52 4	12 4	59 8	9 4
timulatory hold shift	5 2	4 4	4 9	4 7	3 9	2 8	5 2	3 2	6 6	5 2	5 8	6 0
with incomplete *recruitment*												
tation	15 7	13 8	23 1	14 2	27 6	7 7	24 6	15 3	40 3	7 6	22 9	15 0
mulatory balance	56 0	11 0	59 1	8 3	59 0	12 9	61 8	8 9	52 4	11 6	61 1	9 0
timulatory hold shift	6 0	5 6	4 6	4.3	7 8	9 0	6 1	5 6	7 2	4 5	[illegible]	

Ac

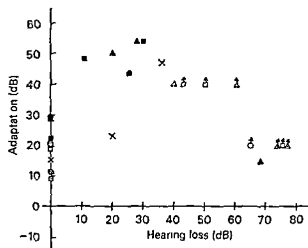


Fig 4 Perstimulatory suprathreshold adaptation on 9 patients with retrocochlear deafness Arrows indicate the loss of the stimulus sensation + = 40 dB, × = 60 dB SL for 500 Hz the squares and the triangles with points 20 dB SL at 1 000 and 2 000 Hz Other symbols as in Fig 2

determination of hearing thresholds Adaptation at 40 or 60 dB SL for 1 000 and 2 000 Hz varied between 18–27 dB, being close to the normal means (Table I)

Mixed group

This group totalled 106 patients (Table I)

The material was divided into two parts, 56 cases with complete recruitment and 50 cases

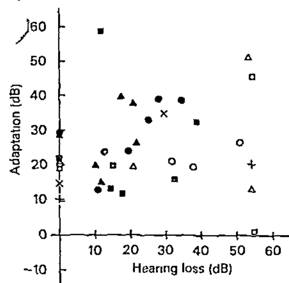


Fig 5 Perstimulatory suprathreshold adaptation in 16 cases with central hearing loss Symbols are the same as in the preceding diagrams

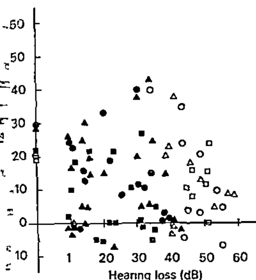
with negative or incomplete recruitm groups included patients from all th etiologic groups

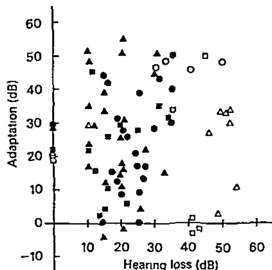
In the first group there were 4 ear threshold tone decay exceeding 30 dE or 4 000 Hz Discrimination scores tween 60–90 % In the second group tone decay exceeded 30 dB in 8 patien discrimination score was lower than ears, in others between 70–90 % In a low speech discrimination score, threshold tone decay was measured Perstimulatory suprathreshold adaptat recruiting ears (Table IV) was statistic to that measured in cochlear lesions ($P<0.05$) than recorded in the norma and in the patients with negative or recruitment excluding the mean of 4 at 1 000 Hz The latter group did not d normally hearing ears The individu are presented in Fig 6

Pronounced threshold tone decay (n 30 dB) coincided with large adaptation in patients with incomplete *tive loudness recruitment* The recru did not show this correlation The tw did not differ statistically as regards *latory balance levels and* post stimula shold shifts, although the means ten higher than in normal material Neithe *threshold amplitudes* (interrupted the excursion widths of prestimulator and adaptation tracings differ from nor

DISCUSSION

Suprathreshold adaptation in end orga has generally been found to be close to even though so high values as were pat *have reported* (Hood, 1950, Palva 195 lozza & Cioce, 1962 Cioce & Spel Manzini et al, 1956) Because of meas techniques used by the above investiga normal values were lower than those by the present author (Kärja, 1968) Ad in primary hair cell lesions in this stud to be less than in the normally hear





5 Perstimulatory suprathreshold adaptation in 56 ns with complete recruitment (*left*) and in 50 cases showing negative or incomplete recruitment (*right*) in the mixed group of sensorineural deafness

onductive deafness (Kärjä, 1970), and it ed to decrease with increasing hearing loss ever, adaptation values comparable to nal were found in some ears with slight ing loss (Fig 2) This must signify that emaining functioning hair cells in the ears cochlear damage adapt less in the case of uli of equal sensation level and of the same d pressure level than do the corresponding ulated hair cells in normally hearing ears

Ieniere s disease, regardless of the degree of hreshold loss, there were several cases with adaptation values (Fig 3) This accords Wood who showed that in most unilateral adaptation in the diseased ear was greater in the healthy comparison ear This excep- il behaviour in Meniere's disease compared other inner ear lesions might be due to the that the hearing loss is caused by a probable rochemical disorder, not by primary hair lamage

he recruiting ears in a mixed group consisting arious types of sensorineural hearing loss ed similarly to the ears with primary hair esions, lower adaptation values as compared the normal material, whereas adaptation in ars with incomplete or negative recruitment indicating a possible retrocochlear lesion tended to be significantly higher The values in the latter group were statistically higher than those for the ears with complete recruitment Suprathreshold adaptation in cases with VIII nerve lesions was abnormally high almost without any exceptions Pestalozza & Cioce have reported comparable results It seems that in these cases the number of the functioning neurons is too slight to transfer continuous acoustic information, the stimulation is accompanied by a pathologic loudness level loss Massive lesion of afferent neurons of this kind is seldom possible on higher levels, and adaptation in central nervous lesions is thus normal

In verified VIII nerve lesions, and in ears with negative recruitment suggesting retrocochlear deafness, perstimulatory suprathreshold adaptation of high degree was associated with pronounced adaptation at threshold level In other etiologic categories no correlation was found between the two tests A considerable loudness level loss could be associated with only a slight adaptation at threshold and cases with slight adaptation or none at suprathreshold level could show any amount of tone decay at threshold

Prestimulatory balance levels of recruiting

ears tended to be higher than in normally hearing material This is apparently due to the recruitment phenomenon, for equal loudness in the control ear the intensity of the comparison tone must be greater than the stimulus intensity in the recruiting test ear

The excursion widths of threshold amplitudes and of prestimulatory balance and adaptation tracings tended to be wider than in the normal material The normally hearing subjects participated in a greater number of test runs and were thus more familiar with the recording technique As is well known, the excursion amplitudes of the threshold tracings registered with continuous test tones are in most cases less than 5 dB for recruiting ears at frequencies 2 000 Hz or more This is not the case with interrupted test tone as used in the present study The reasons may be that the threshold level for a continuous test tone caused by adaptation is poorer than for an interrupted tone and the recruitment phenomenon is more pronounced at higher intensities than close to the threshold minimum

ZUSAMMENFASSUNG

Perstimulatorische überschwellige Adaptation wurde an 243 Patienten mit verschiedener Schallempfindungs schwerhorigkeit gepruft Die Messtechnik grundete sich auf binauralen, dauernden Lautstarkevergleich Der ... fton bestand aus 200 msec langen Impulsen und ... langen Intervallen Stimulusintensität war 20, ... oder 60 dB uber dem Schwellenwert, fur die Fre... von 1 000–4 000 Hz Bei Haarzellenbeschadigungen war die Adaptation geringer als in den normal horenden Ohren Sehr grosse Adaptationswerte wurden bei retrocochleären Storungen gemessen Bei ... Schwerhorigkeit war die Adaptation normal

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MONITORING THE POST-MASTOIDECTOMY STATUS BY MEANS OF RADIOACTIVE STRONTIUM (Sr-85)

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(Received December 13, 1973)

act Stimulation of postoperative calcium metab- in the mastoid process was studied by means of active strontium (Sr 85) in a series of 52 patients underwent an ear operation Calcium metabolism ed maximal values within 3 months began to ase after 6 months and reached the normal level e course of 2 years Patients subjected to ear surgery ld therefore be followed up for at least 2 years operatively before the outcome in respect of osseous ges can be regarded as permanent

use of radioisotopes in the diagnosis of e bone diseases has gained increasing clinical ortance in recent years Bone-seeking isotopes) to locate and determine quantitatively the ulation of bone calcium metabolism induced a local osseous process such as a malignant iour, osteitis or a fracture There is intensive ake of bone seeking calcium, strontium, fluor, at sites of new bone formation As almost osseous processes show new formation in ition to resorption, it is usually possible to monstrate and measure the bony processes h radioisotopes of these substances The ie method may also be employed to w when the osseous process has ended iuma induced local stimulation of calcium tabolism can be established with strontium- within the first few days after a trauma ontium activity is preserved in cancellous ie for 2 years, though decreasing, it may be ained for 6–9 years in hard bones (Bessler, 67 Hall et al, 1967, Wendeberg, 1961, Vittali Merckling, 1970) Strontium activity behaves in the same way after bone operations as after bone fractures (Bessler, 1967)

The strontium-85 measurements we made in cases of mastoiditis (Härmä et al, 1971) led to the application of the method to sequelae of middle ear operations The purpose of this study was to establish with the help of Sr 85 measurement when the bony process after ear surgery has ended in the mastoid process Objective determination of this time point would give at the same time an excellent indication as to how long operations on the middle ear should be monitored clinically before the outcome can be regarded as permanent

MATERIAL AND METHODS

Our material consisted of 52 operated ears Radical mastoidectomy had been performed on 50 of them and limited atticotomy in 2 cases The posterior wall of the auditory meatus had been left intact in 23 patients subjected to 50 radical operations There were 15 cases in which it had been freed temporarily during the operation and been replaced, or the operative cavity had been filled with bone chips chiselled from the cortical layer of the mastoid process Two or more investigations had been made on six ears, making a total of 64 The earliest cases had been measured a few days, the most recent 5 5 years postoperatively The investigation material was so selected that the control ear of

ears tended to be higher than in normally hearing material This is apparently due to the recruitment phenomenon, for equal loudness in the control ear the intensity of the comparison tone must be greater than the stimulus intensity in the recruiting test ear

The excursion widths of threshold amplitudes and of prestimulatory balance and adaptation tracings tended to be wider than in the normal material The normally hearing subjects participated in a greater number of test runs and were thus more familiar with the recording technique As is well known, the excursion amplitudes of the threshold tracings registered with continuous test tones are in most cases less than 5 dB for recruiting ears at frequencies 2 000 Hz or more This is not the case with interrupted test tone as used in the present study The reasons may be that the threshold level for a continuous test tone caused by adaptation is poorer than for an interrupted tone and the recruitment phenomenon is more pronounced at higher intensities than close to the threshold minimum

ZUSAMMENFASSUNG

Perstimulatorische uberschwellige Adaptation wurde an 243 Patienten mit verschiedener Schallempfindungsschwerhorigkeit gepruft Die Messtechnik grundete sich auf binauralen, dauernden Lautstarkevergleich Der Prufton bestand aus 200 msec langen Impulsen und langen Intervallen Stimulusintensitat war 20, oder 60 dB uber dem Schwellenwert, fur die Fre- von 1 000–4 000 Hz. Bei Haarzellenbeschadigungen war die Adaptation geringer als in den normalhorenden Ohren Sehr grosse Adaptationswerte wurden bei retrocochleären Störungen gemessen. Be Schwerhorigkeit war die Adaptation normal.

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MONITORING THE POST-MASTOIDECTOMY STATUS BY MEANS OF RADIOACTIVE STRONTIUM (Sr-85)

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act *Stimulation of postoperative calcium metab-* ι in the mastoid process was studied by means of ·active strontium (Sr 85) in a series of 52 patients underwent an ear operation Calcium metabolism ιed maximal values within 3 months began to ase after 6 months and reached the normal level e course of 2 years Patients subjected to ear surgery ld therefore be followed up for at least 2 years operatively before the outcome in respect of osseous ges can be regarded as permanent

use of radioisotopes in the diagnosis of e bone diseases has gained increasing clinical ortance in recent years Bone-seeking isotopes) to locate and determine quantitatively the ulation of bone calcium metabolism induced ι local osseous process such as a malignant our, osteitis or a fracture There is intensive ake of bone seeking calcium, strontium, fluor, at sites of new bone formation As almost osseous processes show new formation in ition to resorption, it is usually possible to ionstrate and measure the bony processes ι radioisotopes of these substances The ie method may also be employed to w when the osseous process has ended uma-induced local stimulation of calcium abolism can be established with strontium- within the first few days after a trauma ontium activity is preserved in cancellous ie for 2 years, though decreasing, it may be ined for 6–9 years in hard bones (Bessler, 7 Hall et al , 1967, Wendeberg, 1961, Vittali Merckling, 1970) Strontium activity behaves in the same way after bone operations as after bone fractures (Bessler, 1967)

The strontium 85 measurements we made in cases of mastoiditis (Hārmā et al , 1971) led to the application of the method to sequelae of middle ear operations The purpose of this study was to establish with the help of Sr-85 measurement when the bony process after ear surgery has ended in the mastoid process Objective determination of this time point would give at the same time an excellent indication as to how long operations on the middle ear should be monitored clinically before the outcome can be regarded as permanent

MATERIAL AND METHODS

Our material consisted of 52 operated ears Radical mastoidectomy had been performed on 50 of them and limited atticotomy in 2 cases The posterior wall of the auditory meatus had been left intact in 23 patients subjected to 50 radical operations There were 15 cases in which it had been freed temporarily during the opera tion and been replaced, or the operative cavity had been filled with bone chips chiselled from the cortical layer of the mastoid process Two or more investigations had been made on six ears, making a total of 64 The earliest cases had been measured a few days, the most recent 5 5 years postoperatively The investigation material was so selected that the control ear of

Fig 1 Sr 85 uptake (U) in ear[s after] operation (o) and in normal ears [...] mean of normal persons is 0 %, [...] hatched areas 1 S D and 2 S.D [...] mean

$$U = \frac{c - c_o}{c_o} \times 100\ \% \text{ (patients)}$$

$$U = \frac{c_r - c_l}{c_l} \times 100\ \% \text{ (normal persons)}$$

c = counts in operated ear
c_o = counts in healthy ear
c_r = counts in right ear
c_l = counts in left ear

each patient had to be healthy and the postoperative phase of recovery must have been uneventful Cases in which the ear failed to dry post-operatively were excluded The control material was comprised of 20 persons free from ear disease

The measuring method used was a slightly modified version of the one presented earlier (Härmä et al, 1971) The isotope used was Sr-85 as strontium chloride A dose of 25 μCi was injected intravenously 72 hours before the measurement The measurement was made with a conventional detector analyser-scaler device Counts were taken five times on each side sepa-[rately], with the measuring aperture of the detec-[tor] at right angles to the mastoid The measuring [side] was changed after each count The crystal-skin distance was 7 cm and the measuring area on the skin was a circle 5 cm in diameter The measuring time of one count was 1 minute, making a total of 5 minutes on each side The room background (patient present) was subtracted from the counts The result is presented as the difference in counts between the operated and unoperated ears as a percentage of the counts from the unoperated ear In the control material the difference was measured from the counts of the left and right ears (see caption to the figure) This means that in both materials if the uptake in the latter ear is greater than that in the former, the percentage is negative

RESULTS

The results are shown in Fig. 1 S[...] activity rose very sharply in the cour[se of] months The highest values [...] cases in which either the posterior wall [of the] auditory meatus had been freed tempor[arily or] bone chips had been used to fill the [...] cavity Activity began to decrease h[...] regardless of the type of operation perf[ormed] some 6 months after the operation and r[eached] normal distribution in the course of 2 [years] If over 2 years had elapsed from surgery [the] measuring results kept within the ran[ge of] normal distribution Activity remained [...] in the cases in which the ear failed to d[ry ...] the inflammatory osseous process pers[isted in] the mastoid process, but these cases w[ere ex]cluded from the present series

CONCLUSIONS AND DISCUSSIO[N]

The stabilisation of calcium metabolism [...] to take place during 2 years concurs w[ith] clinical experience and follow up stud[ies ...] high incidence of changes in hearing [...] months after ear surgery, even years la[ter has] been reported Palva et al stated in 19[...] half a year after obliteration according [...] method the ear canals were larger than p[...] tively, and this trend continued to the e[nd] point Two years postoperatively the [...] size was the same as at 12 months The [...]

radiograms that new bone was still forming : second and third postoperative year It unlikely that a significant degree of new formation continued in our series over 2 after the operation

: studies published to date on the late s of ear operations have been typical / up investigations There has been a lack arity and some disagreement about the in which the postoperative result must be ded as permanent The method described permits objective detection of the cessation e osseous processes in the operative area ly be claimed on the strength of our own rial that the follow up period for ear opera- must be at least 2 years before the outcome construction of the auditory meatus can be ded as permanent The same is obviously of tympanoplasties, for the position of the ory ossicles and the tension of the tympa- s membrane can probably be regarded as lised only when the calcium activity of the lle ear has subsided As we showed in our er study, increased uptake in the unoperated uggests inflammation in the mastoid process thus possible to show with the method also presence of the bony process in the ear ated on 2 years earlier High activity which sts for over 6 months indicates, similarly, the inflammatory process in the operated oid process has not subsided

ZUSAMMENFASSUNG

Die Stimulation des postoperativen Calciumstoffwechsels im mastoiden Fortsatz wurde mit Hilfe von radioaktivem Strontium (Sr 85) an 52 am Ohr operierten Patienten untersucht Der Calciumstoffwechsel erreicht nach drei Monaten maximale Werte, beginnt nach sechs Monaten zu fallen und zeigt nach zwei Jahren normale Werte Demnach sollten Patienten nach Ohrenopera tionen mindestens zwei Jahre lang postoperativ betreut werden, damit das Ergebnis hinsichtlich Knochenveranderungen als permanent gelten kann

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AN ELECTRON MICROSCOPIC STUDY OF THE TYMPANOJUGULAR GLOMUS

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Abstract Seven temporal bones from four human foetuses of crown–rump length 55–62 mm were prepared for electron microscopy and stepwise sectioned Seven glomera were found along the tympanic and auricular branches and/or in the adventitia of the superior bulb of the jugular vein The glomus parenchyma was made up of chief cells characterized by a content of membrane-bound, osmiophilic granules 114 nm in mean diameter The cells were almost completely invested by supporting cells in which nerve fibres were embedded as well The numerous vessels contained fenestrae in the endothelium. The fine structure of the tympanojugular glomus is similar to that of the carotid body

Tympanojugular glomus is the term used for the complex of small glomera located along the auricular branch of the vagus nerve and the tympanic branch of the glossopharyngeal nerve in relation to the adventitia of the superior bulb the jugular vein and in the middle ear

osenwasser (1945 and 1968) reported the first cognized case of a carotid glomus tumour-like mass occurring in the middle ear He suggested that the tumour had developed from the glomus jugulare described by Guild (1941) So ... knowledge of the morphology of the ... tympanojugular glomus has been based ... microscopy (Guild, 1953, Vara-Thorbeck ... Zettergren & Lindström, 1951) Electron ... scopy is of interest in order to elucidate ... or not the tympanojugular glomus is ... logically similar to the carotid glomus.

MATERIAL AND METHOD

Seven temporal bones obtained from ... man foetuses designed below by their ... rump length in millimetres cr 55, cr 59 ... and cr 62 Under general anaesthesia the ... ses were removed by hysterotomy Th... cord vessel 11–20 ml cold 3% glutaral... buffered with cacodylate to pH 7 4 was ... The area of the tympanojugular glomus ... out The removed specimens were further ... for 7–24 hours by immersion into cold

Fig 1 Light microscopic low power view of a survey section cut parallel to the posterior surface of the petrous portion of the left temporal bone from foetus cr 62 A small glomus (*11*) is embedded laterally in the superior bulb of the jugular vein (*10*) The glomus is not in immediate relation to the auricular branch which is situated in the future mastoid canaliculus (*12*) or to the tympanic branch which is situated on the promontory (*9*) Basal turn of cochlea (*1*) Internal carotid artery (*2*) Superior cervical ganglion (*3*) Glossopharyngeal nerve area peripheral to the ganglia (*4*) Inferior petrous sinus (*5*) Inferior ganglion of vagus nerve (*6*) Accessory nerve (*7*) Loose mesenchyme in the future tympanic cavity (*8*) Tympanic branch of the glossopharyngeal nerve (*9*) Internal jugular vein (*10*) Glomus (*11*) Auricular branch of the vagus nerve (*12*) × 35

Fig 2 Light microscopic micrograph showing ... jugular glomus from foetus cr 62. The ... situated in close relation to the endothelium of ... ternal jugular vein (*5*) The majority of cells are ... to be chief cells (*4*) a few supporting cells (*3*). The ... is very vascular (2) considering its size. × 480.
Fig 3 Electron microscopic low power view of ... panojugular glomus from foetus cr *59 Of the* ... sectioned through the nucleus two may be ... as chief cells (*cc*) with regular, relatively pale ... and one as a supporting cell (*sc*) with an ... shaped nucleus of a dense chromatin *structure*. The ... between the chief cells is of a highly intricate arch... being composed of chief cell parts (*ccp*), nerve ... (*nf*) and thin processes (*arrows*) × 4 000


2
3
4
5
6
1
9
7
8
10
11
12
500 µm


2
2
3
4
5
20 µm


3
cc
cc
cbp
2 µm

Table I *Material, processing, and findings of tympanojugular glomus*

	Foetus cr 55 R side	Foetus cr 55 L side	Foetus cr 59 R side	Foetus cr 61 R side	Foetus cr 61 L side	Foetus cr 62 R side	Foetus cr 6? L side
Stepwise sectioned depth, mm	0 9	1 2	0 8	0 4	1 2	2 0	2 3
No of survey sections	179	94	110	40	171	237	234
Mean distance between sections, µm	4 9	12 8	7 6	10 8	6 8	8 4	9 7
No of ultrapyramids without glomus	1	2	1	1	6	0	0
No of ultrapyramids with glomus	1	0	1	0	0	3	3
Stepwise sectioned parts (in fractions) of the *auricular branch* and	Medial 1/4 in jugular fossa	Medial 1/3 in jugular fossa	None	Medial 1/3 in jugular fossa	Medial 1/2 in jugular fossa	1/1 in jugular fossa 1/1 in mastoid canaliculus 1/2 in facial canal	1 1 in jugul fossa 1 1 in masto canaliculus 2 3 in facial canal
Number of glomera found along or close to the branch	1	0	0	0	0	2	2
Stepwise sectioned parts (in fractions) of the *tympanic branch* and	None	None	1/1 infra tympanic 2/3 intra tympanic	1/1 infra tympanic	None	1/1 infra tympanic 2/3 intra tympanic	1 1 infra tymp 1 2 intra tymp
Number of glomera found along the branch	0	0	1	0	0	0	1

utaraldehyde, rinsed in 0 05 M cacodylate ffer, kept for about 24 hours in sucrose, and ost fixed for 1 hour in 1 % osmium tetroxide The specimens from foetuses cr 55 and 61 were dehydrated in increasing concentrations of ethyl alcohol and carried through propylene oxide to be embedded in Maraglas (Freeman & Spurlock, 1962), specimens from foetuses cr 59 and cr 62 were dehydrated in acetone and carried through propylene oxide to be embedded in Araldite The specimens were placed with the posterior surface of the petrous portion of the temporal bone on the flat lid of Beem plastic capsules No 00 whose pyramid-shaped end, generally used for embedding, had been cut off The blocks were polymerized for 48 hours at 60°C

The blocks were trimmed in part manually with cleaned razor blades and in part with a Reichert TM60 machine, equipped to mesas Survey sections and ultrathin se were cut on a Reichert OmU2 ultramicr mounted with glass knives The ½ µm sections used for light microscopy were s with basic toluidine blue for 1–2 minutes hot plate at about 80°C and mounted in Ultrathin sections were collected on supp copper grids coated with 1 4 % Formvar double-contrasted with zinc uranyl aceta and lead citrate (Venable & Coggeshall, The ultrathin sections were studied and graphed in a JEM-T7 electron microscope

To locate the glomera, specimen blocks stepwise sectioned Each mounted survey s was assessed in the light microscope for c of glomus tissue, and the depth of the nex was based upon this assessment If a s



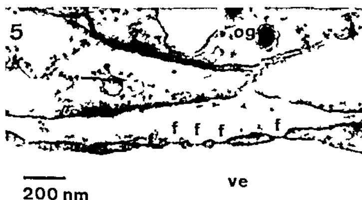


Fig 4 Electron microscopic medium power view showing organelles in a tympanojugular glomus chief cell from foetus cr 67 The chromatin is granular and concentrated predominantly in fragments (*ch*) which are partly scattered in the nuclear ground substance and partly accumulated beneath the nuclear membranes In the cytoplasm Golgi zone (*g*) whose only cistern contains electron opaque material of an appearance like the content of the osmiophilic granules (*og*) mitochondria (*m*) granular endoplasmic reticulum (*ger*) vacuoles with pale content (*va*) dense body (*db*) and cilium (*ci*) × 14 000

Fig 5 Electron microscopic high power view showing endothelium in a tympanojugular glomus vessel from foetus cr 62 A chief cell part with osmiophilic granules (*og*) is separated from the vessel lumen (*ve*) by a non granular cell process (*arrow*) and the fenestrated endothelium (*f*) × 57 000

-ion contained cells suspected of being glomus s a pyramid for ultramicrotomy was trimmed ble I summarizes the processing of the speci n blocks the findings of tympanojugular mera and characterizes the searched areas the temporal bones

Measurements on the micrographs were done h a Zeiss particle size analyser TGZ 3

RESULTS

Seven tympanojugular glomera were found and studied in the electron microscope Two were situated in close relation to the tympanic branch, one in its course between the inferior ganglion of the glossopharyngeal nerve and the tympanic canaliculus and one on the promontory in the

middle ear Two glomera were situated in immediate relation to the auricular branch on its course in the jugular fossa, and three periad ventitially in the superior bulb of the jugular vein without contact with the auricular branch (Fig 1)

In the light microscope (Fig 2) the organs were about 60 × 45 μm, and were made up mainly of cells with rounded, pale nuclei, and ample cytoplasm (chief cells), and a few cells with smaller, dark, irregular nuclei and scanty cytoplasm (supporting cells) Thin walled vessels were numerous, except in glomus cr 55

In the electron microscope (Figs 3, 4, 5) the glomera were made up of vessels, nerve fibres, and two types of cell, granular chief cells and non granular supporting cells The nuclei of the supporting cells were irregular, often semilunar, and relatively dark, surrounded by a narrow rim of perinuclear cytoplasm containing a small Golgi zone and a few ribosomes as well as mito chondria It was characteristic of this type of cell that it gave off thin cytoplasmic extensions em bracing chief cells and/or nerve fibres

The nucleus of the chief was usually rounded and ovoid The chromatin fragments were irregularly distributed, but the chromatin frag ments always accumulated beneath the nuclear membrane and close to nucleoli The peri clear cistern was closed in punctate spots by lear pores The mitochondria were oval or shaped with transversely placed crests and a few granules in the matrix The rough endo plasmic reticulum was scanty, occurring partly in the form of solitary, flat vesicles and partly as a few parallel lamellae Similar small vesicles and lamellae had no ribosomes attached to their outer surface Non membrane bound ribo somes occurred in major numbers usually collected in small groups The cytoplasm contained lysosome like bodies of different types and micro tubules Cilia of a 9 × 2 + 0 structure were observed in a very few cells in whose cytoplasm they were deeply invaginated Where two chief cells adjoined, desmosome-like structures might occur The striking feature was vesicles which at some distance surrounded an electron dense, almost homogeneous, central core These nules showed quite some variation in appea and size The mean diameter in 837 gra measured was 114 nm, standard deviation

In all glomera nerve fibres were pro Major bundles were observed even in low p views, and at higher magnification num nerve fibres were seen to be wedged in be chief and supporting cells No synapses seen

The exact mutual relation between chief supporting cells, and nerve fibres was di to interpret, especially in areas predominate cell processes This was due partly to the r cate architecture and partly to the consta sence of cell processes which were difficult identify As a main rule, chief cells either joined other chief cells or else were surro by thin, non granular cell processes It was in very limited areas that the plasma mem of the chief cells was in direct contact wi endothelium of the vessels, nerve fibres, or wide intercellular spaces

The glomic vessels consisted of a thin l of endothelial cells, partially surrounded b vascular cells The endothelium contained of fenestrae The vascular lumina were separated from the chief cells by pericytes as by supporting cells, but a closer contact also occur

DISCUSSION AND CONCLUSION

Based on light microscopy Guild (1953) s that the histological structure of tympano glomus was like that of the carotid body however, stressed that his method of prepar of the temporal bone material imposed limitations on this conclusion Compared the fine structure of the carotid body human foetuses (Kjærgaard, 1973) the st electron microscopic cytological details of tympanojugular glomus revealed no ess morphological differences ' No charact has been found by which, under the micro a section from one of these structures (i e panojugular glomera) can be distinguished f

tion of the other (i e carotid body)" Guild e In the era of the electron microscope is still valid

ZUSAMMENFASSUNG

n Schlafenbeine von vier menschlichen Foten itel-Steiss Lange 55–62 mm) wurden fur die Elektroikroskopie praparıert Es wurden sieben Glomera den, die in der Nachbarschaft des N tympanicus les R auricularis und/oder in der Adventitia des is venae jugularis superior lagen Das Glomuschym bestand hauptsachlich aus Hauptzellen, kterisiert durch ihren Gehalt an osmiophilen ula mit einem durchschnittlichen Vesikeldurch r von 114 nm Die Zellen waren fast vollständig tutzzellen eingescheidet die auch Nervenfasern elten Das Endothel der zahlreichen Gefasse war triert Die Feinstruktur des Glomus tympanoju e gleicht der des Glomus caroticum

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RESPONSES OF RESISTANCE AND CAPACITANCE VESSELS IN FELINE NASAL MUCOSA TO VASOACTIVE AGENTS

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(Received December 19 1973)

Abstract Blood flow from the mucosa of the right nasal cavity was measured with a flowmeter connected to the vein passing through the pterygopalatine foramen in cats [illegible] changes

same cavity These two methods could provide information about responses evoked in the resistance vessels (mainly small arteries and arterioles) and in the capacitance vessels (mainly venous vessels and sinusoids) Close arterially administered infusions of angiotensin noradrenaline adrenaline and dihydroergotamine elicited constrictions in the resistance as well as the capacitance vessels Analysis of the ratio of the resistance and the capacitance responses permitted an evaluation of the relative effectiveness of the drugs in constricting the two types of vessel The constrictor action on the capacitance vessels was in the order Dihydroergotamine > the catecholamines > angiotensin The order was reversed as ds the constrictor action on the resistance vessels

)measurements of blood flow, arterial and ous pressure, and changes of tissue in various regions, such as skeletal muscle, skin, or intestine it has been possible to analyse in detail the various peripheral vascular functions, i e of the resistance vessels with a precapillary and a postcapillary section, the precapillary sphincter vessels, the capillary exchange vessels, the capacitance vessels and, in some tissues, the shunt vessels (Mellander & Johansson, 1968, Folkow & Neil, 1971) The same vascular functions seem to be present in the nasal mucosa Previous studies describe effects of autonomic nerve stimulation and drug application on muc nous blood flow and patency in of the cat, the latter measured by a ballo nique (Malm, 1973*a* and *b*) In the prese a critical analysis was performed to in to what extent regional blood flow and recordings combined with this balloon te might provide direct information about t tions in the resistance and capacitance of the nasal mucosa as evoked by certa active agents

METHOD

The results were obtained from experi cats, which were anaesthetized with chl (about 80 mg/kg i v) after induction wi Tracheotomy was performed to facilitat taneous respiration Venous blood flo changes in nasal patency were recorded ciple as described previously (Malm 1973 some recent modifications In brief, the mental approach was as follows The c lying on its back with its mouth partly a fixed position To reach the vein whi the nasal mucosa through the pterygop foramen, the mucosa of the hard palate sected free and part of the underlyi drilled away A thin polyethylene cathet diameter 0 6 mm, length 2 cm) was inser the vein near the dorsal surface of the t right molar tooth and carefully advance vessel until the tip of the catheter was o

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from the pterygopalatine foramen The vein ligated around the catheter close to the men It was connected to another catheter rger bore (inner diameter 1 7 mm, length n), diverting the venous blood flow through ptical blood flow recorder unit, after which is returned to the animal via a funnel con- :d to the right jugular vein Blood flow was nuously recorded on kymograph paper The it of the venous outflow catheter was usually t a level 6 to 8 cm above the pterygopalatine nen, but could be adjusted if desired (see w) Changes of nasal patency were estimated rms of pressure changes in a water filled ion placed in the right nasal cavity The on was connected via a polyethylene tubing n electronic pressure transducer (EMT 33, ia-Schonander) for recording on a poly- h (Mingograph, M81, Elema-Schonander, [illegible]

essure bottle and the system was then closed rial blood pressure was recorded from the t femoral artery via a mercury manometer he kymograph Close arterial infusions were n through the right lingual artery in retro- le direction via a polyethylene catheter The were heparinized just before the insertion he catheters Early in the experiment the t cervical sympathetic nerve, and as a rule the right vidian nerve, were cut

he following drugs were administered close rially to the nasal mucosa through the lingual y as described, via a constant infusion ip *l* adrenaline bitartrate and *l* noradrenaline rtrate (0 001, 0 01, 0 1 and 1 μg/kg b w ×), angiotensin-II-amide (Hypertensin N®, a) (0 001, 0 01, 0 03 and 0 1 μg/kg b w ×), and dihydroergotamine-methan-sulpho- (Orstanorm®, Sandoz), (1 and 10 μg/kg b w nn)

lata are given as mean values ±S E M Stu t s *t* test was used for statistical evaluation

RESULT

Evaluation of Techniques for Recording Resistance and Capacitance Functions

Venous blood flow at rest

It was essential to define first the region from which blood was drained by the present catheterization technique and this was done by the following procedures Towards the end of the experiment, or when the cat was dead, infusions of a dye (methylene blue) or of a roentgen contrast medium (barium sulphate) were given in retrograde direction through the venous outflow catheter to study the vascular distribution of these tracer agents either by direct inspection or by angiograms When the tracers were given under a slight infusion pressure without marked inflow resistance they were distributed almost exclusively to the nasal mucosa of the right cavity, as can be seen in Fig 1A (barium sulphate) With a somewhat higher infusion pressure which overcame a certain inflow resistance, the solutions were forced over to the mucosa of the left cavity and its major draining vein, as demonstrated in Fig 1B The methylene blue method gave similar results The conclusion is that the right pterygopalatine vein drains blood from the nasal mucosa alone, and normally only from that of the right cavity It cannot be excluded, however, that a fraction of the mucosal blood flow on the right side is drained by some other small veins, the extent of which may depend on venous outflow pressure

Blood flow rates were therefore measured at different levels of venous outflow pressure in some cats It was established that at each given level of venous outflow pressure the blood flow rate was constant throughout prolonged observation periods It was also found that the blood flow rate was much faster at the low venous outflow pressures than at the high The blood flow rate was, for instance, about twice as big when the venous outflow catheter tip was set at the level of the nasal cavity (approximately heart level) as when the outflow catheter was 6 to 8 cm above heart level It must be added that the resistance in the venous outflow catheter was

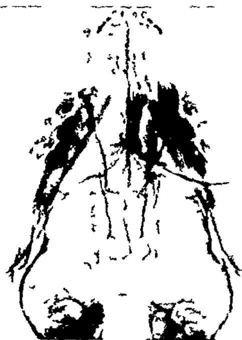


Fig 1 (A) Angiogram of the scull of a cat (submento vertical projection) after barium sulphate injection through a catheter inserted into the vein passing the right pterygopalatine foramen The opaque medium is distributed almost only to the nasal mucosa of the right cavity



Fig 1 (B) Angiogram after barium sulphate administration applied at higher injection pressure than in Fig 1A The medium is forced into a larger area of the mucosa on the right side and also to the left cavity and to the vein passing the left pterygopalatine foramen

not entirely negligible but caused a of 1 to 3 cm H_2O at the blood flov countered in the present study

The normal pressure in the veins was estimated to be about 7 cm H_2O flow rates were therefore measured of cats with the outflow catheter 6 to heart level In these experiments nas was also measured by the balloon tech application of a high venous outflo here also served to prevent the s venules and the veins from being by the water pressure (4 cm) in th which is important for the study o pacitance responses (cf Öberg 1967) U circumstances, the venous blood flow 0 6±0 1 ml/min (6 cats) It is possib higher flow value at the low venou pressure can be explained by blood from the mucosa not only in the righ in the left cavity The flow value at t outflow pressure of 6 to 8 cm H O r fore more truly reflect flow from the the right cavity alone Since the wet the mucosa in the right cavity, determ careful dissection was found to a ±0 04 g, resting blood flow in the nas can be estimated to be in the range c ml/min × 100 g tissue

Estimation of changes of mucosal tissue

A change of tissue caused for insta change of regional blood volume response), is considered to be represe pressure change in a balloon inserted cavity This conclusion was based on th ing model experiment tests in situ

A model of the nasal cavity and of s to that in the cat was designed for th balloon technique The model consis latex tube surrounded by a glass tube what larger diameter The latex and tubes were connected to each other at and the interspace (simulating the nasal was filled with water The volume of t could be changed by injections of wa a syringe, the plunger of which was a

a micrometer screw A water filled balloon, nted around a polyethylene catheter with iections to the latter designed for cat nose :ies (for details see Malm, 1973*a*), was tioned inside the latex tube Balloon pressure ie control period was set to 4 cm H_2O (as in cat experiments) Pressure variations inside balloon were followed via the catheter as the me in the mentioned interspace (model il mucosa) was altered A volume change in latter of 0 016 ml was found to cause a pres- change of 1 cm H_2O in the balloon and there a linear relationship between volume and sure changes within the range of pressure ations observed in the cat experiments (±3 H_2O, see below)

ne balloon technique was also tested in vivo studying the effects of abrupt changes in onal venous pressure causing passive capa- ice responses The cat was then placed on t table and the vascular bed of the nasal osa, the vein through the pterygopalatine men being intact, was exposed to given short- i hydrostatic loads by adjustments of the ht of the nasal region below heart level In e experiments balloon pressure increased increasing hydrostatic load (i e regional id volume) and vice versa

Effects of Drugs

first series of experiments (8 cats) the vascu effects of angiotensin, noradrenaline, and naline were compared when given close arte- y to the nasal mucosa as short term graded sions of 1 min duration Three to 4 different s of each drug were given in each experiment in the ranges mentioned in the method sec-

suprathreshold doses, these drugs always ed a decrease of regional venous blood flow, occasionally when the larger doses were n an increase of arterial blood pressure The st dose (0 001 μg/kg b w) was often sub- shold with regard to the vascular response noradrenaline and adrenaline, but usually not angiotensin Regional vascular resistance expressed in the conventional way in pe- ripheral resistance units (perfusion pressure, mm Hg/regional venous blood flow, ml/min) and the responses of the resistance vessels to the drugs as the per cent change of vascular resist- ance from the control value before drug ad ministration

The nasal patency was always increased by suprathreshold doses of the drugs, as evidenced by a fall of pressure in the balloon in the nasal cavity Such pressure changes, as will be dis- cussed, can be taken as a measure of evoked constrictor responses in the capacitance vessels of the nasal mucosa They may reflect such effects in at least a semi quantitative way since, as indicated by the model experiments, the pres- sure changes were linearly related to evoked changes of cavity volume The elicited capaci- tance responses can be expressed in terms of pressure changes in the balloon (cm H_2O)

Fig 2 (A & B) shows the mean resistance and capacitance responses for all infusions of angiotensin, noradrenaline, and adrenaline when given in suprathreshold doses The amount of angiotensin for all infusions averaged 0 04 μg/ kg b w , of noradrenaline 0 36 μg/kg b w and of adrenaline 0 26 μg/kg b w With this compila tion of data, the *average* constrictor responses of the resistance and capacitance vessels were not significantly different for the three agents To evaluate in greater detail the two vascular responses in relation to each other and to the varying amounts of the drugs, the constrictor effects were expressed as the ratio between the resistance response (per cent increase of resist- ance above control) and the capacitance re- sponse (cm H_2O pressure fall in the balloon) With such a representation, the relative effective- ness of each drug on the resistance and capaci- tance vessels can be revealed The higher the numerical value of the ratio, the greater the rela tive constrictor action of the drug on the resist ance vessels compared to that on the capaci- tance vessels The mean value for these ratios are shown in Fig 2C There was a significant differ ence between the ratios for angiotensin and nor- adrenaline ($p<0 05$) for angiotensin and adrena line ($p<0 01$), but not for noradrenaline and adre-

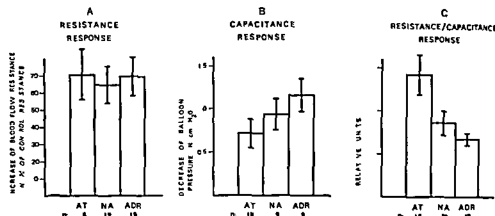


Fig 2 Mean values (±S E M) of the responses in the resistance (A) and the capacitance (B) vessels after infusions of angiotensin (AT) noradrenaline (NA) and adrenaline (ADR) observed in 8 cats The ratio ance/capacitance responses to the three drugs in panel C *n* indicates number of observations

naline ($p>0\ 2$) This implies that angiotensin is a relatively effective constrictor of the resistance vessels, but relatively poor constrictor of the capacitance vessels in the nasal mucosa in comparison with the two catecholamines

A similar comparative study on the nasal mucosa was performed with noradrenaline and dihydroergotamine (9 cats, 5 of which were the same as in the above-mentioned series) Noradrenaline was administered as described and always given before dihydroergotamine The latter substance was given once to each cat as an infusion of duration of 1 to 6 min and in total dose varying from 1 to 10 μg/kg b w ese doses of dihydroergotamine were all rathreshold

Fig 3 shows such an experiment with noradrenaline infusions and one dihydro- mine infusion It can be seen that the cons responses of the resistance and capa vessels to suprathreshold doses of norad (0 1 and 1 μg/kg b w) were evoked rap ing, and disappeared rapidly after the whereas the constrictor responses to d ergotamine developed more gradual and more prolonged, especially in the cap vessels Note that in equi resistant doses droergotamine (*d*) evoked a more pro capacitance response than noradrenaline

Fig 4 (A & B) shows the mean resista capacitance responses for all infusions of adrenaline and dihydroergotamine in th

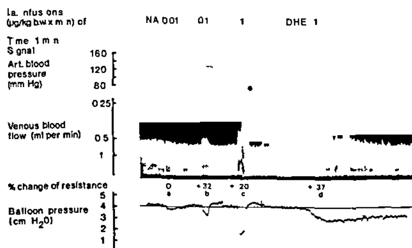
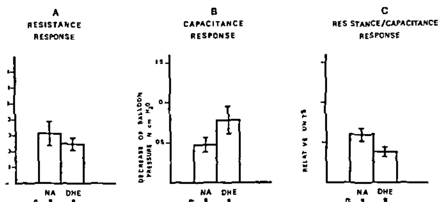


Fig 3 Cat 3 0 kg Effe infusions of noradrenaline dihydroergotamine (DHE) terial blood pressure blood flow vascular resist balloon pressure in the nas A decreased balloon pres measure of increased nasa in turn due to constric capacitance vessels.



. Mean values (+S E M) of the responses in the ...ance (A) and the capacitance (B) vessels after infu... of noradrenaline (NA) and dihydroergotamine (DHE) observed in 9 cats The ratios of resistance/capacitance responses to the drugs are shown in panel C *n* indicates number of observations

...periments The average dose of noradrena... was 0 07 μg/kg b w and of dihydroergota... 5 2 μg/kg b w When the data were com... in this way, no significant difference could ...vealed between the two drugs as regards ... effects on the resistance or capacitance ...ls Yet, the ratios of the resistance and ...citance responses calculated for the various ...ion experiments were found to be different ... 0 05) for the two drugs (Fig 4C) This im... that dihydroergotamine is a relatively more ...tive constrictor of the capacitance vessels ... is noradrenaline

DISCUSSION

...vascular bed of the nasal mucosa differs in ... respects from the vascular design in most ...r regions Thus, besides the conventional ...apillary and postcapillary resistance vessels, ...apillary sphincters, capillary exchange ves... and capacitance vessels, there are arterio...ous anastomoses (Cauna, 1970) and sinus..., the latter probably positioned between ...pithelial capillaries and a deeper venous ...us (Ingelstedt & Rundcrantz, 1963) The ...soids contain smooth muscles in their walls ...nesrekasi, 1969) Filling of the sinusoids ... blood apparently influences nasal patency, ... since such congestion can be pronounced, they must, together with the venous vessels, contribute in an important way to the vascular capacitance function of the nasal mucosa

The resistance function controlling regional blood flow and the capacitance function controlling regional blood volume of course are important determinants of nasal mucosa function There seems to be no investigation from other laboratories in which blood flow in the nasal mucosa has been studied directly, but there are several studies in which changes of nasal patency have been determined (cf Malcomson, 1959) In the present study an attempt was made to follow the resistance and capacitance functions simultaneously and quantitatively in the cat nasal mucosa, and to analyse the patterns of vascular response evoked by certain vaso active agents

The present cannulation technique of the vein passing through the right pterygopalatine foramen seems to permit measurement of blood flow from the nasal mucosa exclusively, although the absolute figures for resting blood flow can only be considered approximate (130 ml/min × 100 g tissue) due to possible minor drainage by other small veins and to the presence of some venous connections with the contralateral nasal cavity The fact that venous outflow at rest remained constant throughout prolonged observation periods at a given venous outflow pressure (see above) and the high reproducibility of the resist-

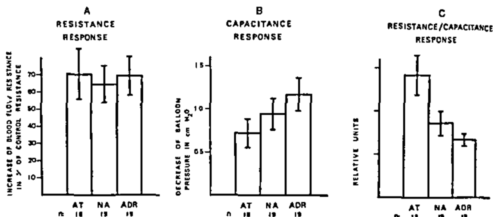


Fig 2 Mean values (±S E M) of the responses in the resistance (A) and the capacitance (B) vessels after infusions of angiotensin (AT), noradrenaline (NA) and adrenaline (ADR) observed in 8 cats The resistance/capacitance responses to the three drugs in panel C. *n* indicates number of observations

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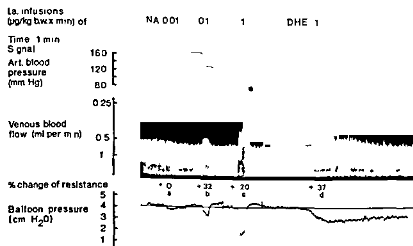


Fig 3 Cat, 3.0 kg. Effe infusions of noradrenaline dihydroergotamine (DHI terial blood pressure, na blood flow, vascular resi balloon pressure in the na A decreased balloon pre measure of increased nas in turn due to constric capacitance vessels

sionen von Angiotensin, Noradrenalin, Adrenalin Dihydroergotamin in der Nahe der Nase verursachten Zusammenziehen sowohl in den Resistanz als auch en Kapazitanzblutgefassen An Hand des Verhaltens nte der relative Effekt der Substanzen auf das Zusammenziehen beider Arten von Blutgefassen beurteilt len Die zusammenziehende Wirkung auf die Kapazi blutgefasse ubten in dieser Reihenfolge Dihydrotamin > die Katekolamine > Angiotensin aus Fur Resistanzblutgefasse war die Reihenfolge umgekehrt

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ance responses to a given pharmacological stimulus, indicate that the method permits quite accurate quantitative recordings of *changes* in mucosal vascular resistance

As evidenced by the model experiment and tests in vivo, the present balloon technique seems to provide information, at least in a semi-quantitative way, about nasal patency as affected by responses of the capacitance vessels in the nasal mucosa This was further corroborated by the fact that the capacitance response to a given pharmacological stimulus was highly reproducible Changes of nasal patency evoked by physiological and pharmacological stimuli have previously been measured by volumetric recordings of air displacement from or to the nasal cavities after closure of the epipharynx and the nostrils (Tschalussow, 1913, Jackson, 1939) In comparison with the present method, the mentioned technique was found less reliable in that baseline drift frequently occurred, probably due to insufficiency of the cavity closure, the reproducibility of the results was also poorer (Malm, unpublished observations)

Nasal patency can be affected not only by *capacitance responses, but also by transcapillary* fluid filtration or absorption and by mucosal secretion Transcapillary fluid movement usually is a relatively slow process (cf Mellander & ohansson, 1968) and, if present at all in these hort term observation periods during drug infusion, such an effect must have been small compared with the observed relatively pronounced capacitance responses Mucosal secretion was apparently negligible in the present experiments with vasoactive agents, since balloon pressure was found always to return to the control level after cessation of the angiotensin, noradrenaline, and adrenaline infusions No secretagogue effect of dihydroergotamine has been *described It may* be concluded that the acute effects observed with the balloon technique can be ascribed to changes in the capacitance vessels

The present study showed that angiotensin, noradrenaline, adrenaline and dihydroergotamine all constricted the resistance as well as the capacitance vessels in the nasal mucosa The analysis of the ratios of the responses of th types of vessel led to the conclusion th relative effectiveness of the substances i creasing vascular resistance may be listed order Angiotensin > the catecholam hydroergotamine The relative effectiven regard to the constrictor response in the tance vessels, on the other hand, appear Dihydroergotamine > the catecholam angiotensin The investigation thus reveal the patterns of vascular response in th series-coupled sections evoked by the f active agents were differentiated and, in the same way as previously described vascular beds of skeletal muscle and sk lander, 1960, Folkow et al, 1961, Mell Nordenfelt, 1970, Jarhult, 1971)

Vasodilatation of the capacitance vess a consequent increase of blood content nasal mucosa is probably one importan factor in decreased nasal patency in the present study therefore may provide a th basis for suggesting that dihydroergotam its preferential and strong constrictor a the capacitance vessels in doses much s *than those causing a* receptor blockade have a beneficial effect in states of nasal tion Clinical research is required to ass a possible therapeutic effect of the dru

ACKNOWLEDGEMENT

The author is indebted to Ass Prof T Olin, De of Diagnostic Radiology, University Hospital, made the angiograms

ZUSAMMENFASSUNG

Nach regionaler Sympathektomie wurde die tung der Mukosa im rechten Nasenraum von einem Stromungsmesser angeschlossen an d Foramen Pterygopalatinum passierende Vene Ausserdem wurde in den gleichen Nasenraum gefullter Ballon eingefuhrt der Druck übertragen konnte Diese beiden Methoden es gleichzeitigen Veranderungen folgen zu somit Aufschluss uber das Geschehen in den blutgefassen (hauptsachlich kleine Arterien und len) und in den Kapazitanzblutgefässen (venose Blutgefasse und Sinusoide) zu erhalten

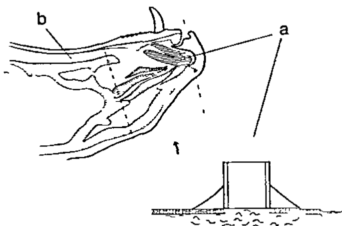


Fig 1 Sagittal section through the head of the cat. Area seen by the detector delineated by dotted lines. Arrow indicates the detector (*a*) The polyethylene tube fixed over the de-epithelized area on the maxilloturbinal (*b*) Nasopharynx.

s in nasal patency following Vidian nerve ation A superior cervical ganglionectomy ed in a complete ipsilateral sympathectomy nasal mucosa of the cat (Änggård & Den- 1974) subsequent stimulation of the Vidi- erve would thus activate parasympathetic fibres selectively

the present study, this finding was exploited he effects of graded parasympathetic nerve ation on the vascular exchange and capac- function in the nasal mucosa were stud- imultaneously using a technique described ously (Änggård & Edwall, 1974) The secre- response in the maxilloturbinal area was studied The aim of the study was to find f the vascular functions are affected by ympathetic nerve activation and whether ecretory response is accompanied by an ased local blood content The frequency– nse relations for these parameters were ed by graded parasympathetic stimulation iermore, a pharmacological analysis of the ilar responses was made

METHODS

riments were conducted on 12 cats weigh- 2–3 5 kg and anesthetized with chloralose– iane (50 mg/kg + 100 mg/kg i v) A supe- cervical ganglionectomy on one side had performed 1 or 2 weeks previously, and the Vidian nerve was purely parasympathe- tic The Vidian nerve was exposed by a trans- orbital approach The eyelids were removed and the eyeball opened and emptied Periorbital dissec- tion and lateral displacement of the eye exposed the Vidian nerve emerging at the apex of the orbit leading to the spheno-palatine ganglion The nerve was transected at the apex and freed from surrounding tissues Minor nerve anasto- moses between the ganglion and the trigeminal nerve were cut Electrical stimulation of the distal end was performed using a bipolar platinum elec- trode The nerve and the electrode were covered with Plastibase (Squibb) Monophasic, square wave pulses with a duration of 1 msec and an inten- sity of 8 V were delivered by a Grass model S 4 stimulator The trachea was cannulated The pressure in the femoral artery was measured via a Statham pressure transducer (P 23 A) and re- corded on a Rikadenki multichannel recorder Rectal temperature was kept constant at 38°C by means of heating lamps The posterior auri- cular artery was cannulated for close intra arterial infusion into the external carotid artery The head of the cat was immobilized by means of a steel rod inserted between the jaws and secured in place by dental acrylic

Measurements of tracer disappearance and local blood content

The technique used to measure changes in the microcirculation was the same as that described in a previous paper (Änggård & , 1974

In essence, changes in the exchange and capacitance function were studied by monitoring the local disappearance rate of an easily diffusible tracer, $^{125}I^-$ and the changes in the gross pulse rate from i v administered $^{131}I^-$ labelled serum albumin

Local disappearance measurements were performed in all experiments ^{125}I as iodide, was obtained as an isotonic carrier-free solution containing 300 $\mu Ci/\mu l$ (AB Atomenergi, Nyköping, Sweden)

The epithelium over a small area of the maxilloturbinal mucosa was removed and a small polyethylene tube (PE 90) applied and fixed over the exposed area with an adhesive (Nobecutan, Bofors), thus creating an open well with the subepithelial tissues in the bottom (Fig 1) One hour later, about 0 3 μl of the tracer solution was placed in the well The disappearance of the applied depot was monitored by an external scintillation detector A 5 cm thick lead shield fitting tightly around the head and the nose of the cat had a narrow slit over the right side of the nose and shielded the rest of the cat from the detector (Fig 1) The detector output was fed into two recording channels, each containing a single channel pulse height analyzer and two scalers with digital printout Radioivity was counted for periods of 40 sec and , started immediately after the lication of the tracer After each run the final background was determined The net pulse rate (gross pulse rate minus background) was plotted semilogarithmically against time The disappearance rate (k value), which represents the fractional elimination of the depot per minute, was calculated as follows (Kety, 1949)

$$k = (\log C_1 - \log C_2)/0\,4343\,(t_2 - t_1)$$

where C_1 and C_2 are the recorded net pulse rates of the depot at times t_1 and t_2 The time interval t_2-t_1 is expressed in minutes The calculations for each time interval were carried out using a computer

In 10 cats changes in local blood content of the nose were studied simultaneously with the disappearance measurements ^{131}I-albumin, around 2 mCi in 5 ml, was injected, intra ly Gross pulse rate was measured over th nasal cavity as described above Appr corrections were made for the ov ^{131}I in the ^{125}I channel For further details see Änggård & Edwall (1974)

Measurements of nasal secretion

Nasal secretion was estimated by inspecti the well formed by the polyethylene tube in the disappearance measurements (Fi Using a Zeiss operation microscope it was sible to observe the accumulation of very quantities of secretion When ... measurements were performed secretion measured semiquantitatively – graded as s (+), noticeable +, obvious ++ or rich -

In three experiments the secretion during s ulation was measured quantitatively Ch in local blood content were recorded sim neously as described above In these studies epithelium in the bottom of the well was removed The volume required to fill the was measured, and the secretion during stimulation period was measured in relat this volume When secretion was rich th required to fill the well was measured was expressed as μl/min

RESULTS

The effects of parasympathetic nerve stim on secretion

In all experiments stimulation frequencies 0 5 or 1 0 imp/sec resulted in a slight secretion Above 2 5 imp/sec secretion was rich, sometimes resulting in an overflow the nasal cavity When secretion was mea quantitatively (3 cats, 31 stimulations) quency-dependent secretion was seen fro to 12 imp/sec, together with simultan creases in gross pulse rate The results representative experiment are shown in Fi

During disappearance measurements s tion could not be evaluated quantitatively volume of the applied tracer solution vari the various applications A rough esti

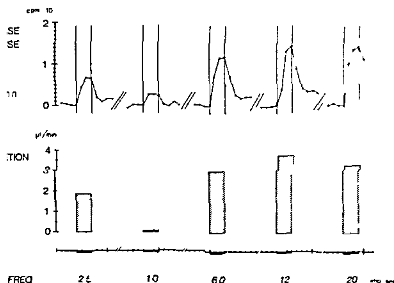
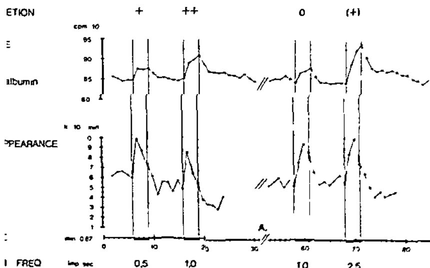


Fig 2 Influence of graded parasympathetic nerve stimulation on the secretory response, and the gross pulse rate of ^{131}I albumin measured over the right nasal cavity

cretion was possible, however In all cases secretion occurred a concomitant increase isappearance rate was seen (Fig 3)

ts of parasympathetic nerve stimulation on disappearance rate and gross pulse rate of lbumin

ulation of parasympathetic fibres to the mucosa with frequencies from 0 5 imp/sec *stently resulted in an increase in ^{125}I- dis*arance rate (*k*-value) and gross pulse rate of ^{131}I albumin (4 procedures) As secretion interfered with the tracer disappearance measurements, a frequency–response relation could not be investigated Fig 3 shows a typical experiment where both tracers were studied at the same time as the secretion A 60% increase in the *k*-value was seen at 0 5 imp/sec Subsequent stimulation with 1 0 imp/sec did not result in a further increase After an initial increase in the *k*-values *during stimulation, a decrease was often noted* This decrease was reduced after administration



3 Influence of parasympathetic nerve stimulation racer disappearance rate of ^{125}I and the gross pulse of ^{131}I albumin measured over the right nasal cavity The secretory response is indicated above the curves None 0 Slight (+) Noticeable + Obvious + + 2,4 kz. (A) Intra-arterial infusion of atropine

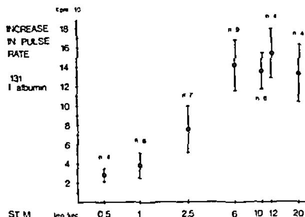


Fig 4 Influence of parasympathetic nerve stimulation on the gross pulse rate of ^{131}I albumin measured over the right nasal cavity Results obtained from 10 cats Mean standard deviation and number of observations are shown

of atropine When secretion occurred on stimulation a reduction of the k values after the stimulation was observed and at stimulation frequencies above 2 5 imp/sec the secretion made dis appearance measurements unreliable After blocking secretion with atropine these effects were not seen (Figs 3 and 5) Therefore it seems that the reduction in disappearance rate during or after stimulation was due to a dilution of the tracer depot by accumulated secretion A leakage of the tracer from the well to areas where the ium had not been removed might also to this effect

Measurements of the local blood content, as studied by ^{131}I albumin showed frequency de-pendent increases in gross pulse rate neously with responses in disappeara (Fig 3) In three experiments stimula quencies between 0 5–20 imp/sec were stimulations) and frequency dependent were seen in gross pulse rate (Figs 2 an 0 5 imp/sec a clear increase was always and major changes in gross pulse rate in the frequency range 0 5–6 imp/sec

To investigate the maximal effects sympathetic and sympathetic tively on gross pulse rate a maximal pathetic stimulation was compared to th mal constriction seen after local applic a vasoconstrictor drug, oxymetazoline Nesenl Draco) In four experiments found that the decrease in gross pulse 3–4 times greater following local appl as compared with the increase seen on parasympathetic stimulation

Effects of atropine, bradykinin and on secretion, tracer disappearance rate blood content

Intra arterial infusion of atropine in doses (1 mg/kg) almost completely bl secretory response following paras stimulation in the range 0 5–20 imp/sec ever, atropine had no obvious effect changes in tracer disappearance rate pulse rate of ^{131}I albumin A repres experiment is shown in Fig 3

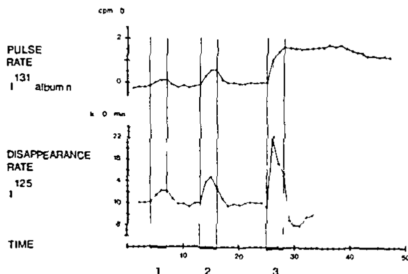


Fig 5 Influence of pa thetic nerve stimulation a kinin on gross pulse rate bumin measured over the r cavity and disappearance ^{125}I Before starting the ex secretion was blocked by 1 mg/kg Cat 3 5 kg (1) S 8 V, 1 msec 2.5 imp/sec ulation 8 V 1 msec 6.0 (3) Intra arterial infusion kinin 0 4 µg/min and 0 resp

completely blocking secretion with atro-, intra-arterial infusion of bradykinin (0 1–ıg/min) resulted in a considerable increase isappearance rate The maximal effects exed those seen after intra-arterial admini-ıon of papaverine and resembled the effects following nerve stimulation (Fig 5)

o investigate whether or not prostaglandins ıt be involved as mediators of the vasodila responses, arachidonic acid (12 mg, at -0 5 mg/min), a prostaglandin precursor, infused intra-arterially in three experiments ... were noted on local blood content or ppearance rate Furthermore, intra-arterial ısion of indomethacin (30 mg), an inhibitor ›rostaglandin biosynthesis, had no effect on ıs pulse rate or disappearance rate following ısympathetic stimulation

DISCUSSION

the present study stimulation of the para ıpathetic fibres in the Vidian nerve resulted ncreases in local blood content, secretion and ıppearance rate of a water-soluble tracer, . from the nasal mucosa, indicating a vaso-ıtory response as well as an enhanced tissue-od exchange

The rates of tracer disappearance have not n transformed into total blood flow values e changes in capillary exchange surface area, ıllary permeability, capillary flow distribution velocity are of quantitative importance and y influence the results (cf Mellander & Johans-, 1968). The arteriovenous anastomoses with-he nasal vascular bed, as described by Dawes Prichard (1953), would also invalidate calcula-ıs of total blood flow based on *k*-values, since ıe are dependent on blood flow through the ex-nge vessels (Kety, 1960) For example, a redi-ıbution of blood flow from shunt vessels to ex nge vessels without changing total blood flow ıld increase the *k*-values Furthermore, the ultaneously induced secretion interfered with measurements and made quantitative evalua ıis unreliable However, increases in disappea-ce rate indicate an increase in the tissue-od exchange

A vasodilatory response within the nasal mucosa in response to stimulation of the Vidian nerve has previously been demonstrated indirectly in rhinomanometric studies (Tschalussow, 1913, Blier, 1930, Malcomson, 1959, Malm, 1973) In these studies changes in the lumen of the nasal passages are recorded and the associated increase in secretion will affect the measurements (Drettner, 1963) With the present technique using $^{131}I^{-}$ labelled serum albumin this error is avoided and a frequency-dependent increase in the local blood content during parasympathetic stimulation was demonstrated Furthermore, in contrast to most studies, a chronic sympathetic denervation had been performed, which prevented activation of the sympathetic vasomotor fibres in the Vidian nerve (Änggård & Densert, 1974)

It is probable that under physiological conditions all autonomic fibres have a low discharge rate, around 1–2 imp/sec (Folkow, 1955) At present, no information is available about the physiological para sympathetic discharge rate to the nasal mucosa Eccles & Wilson (1973) studied nasal secretion during stimulation of parasympathetic fibres in the Vidian nerve without previous degeneration of the sympathetic fibres Stimulation induced a frequency-dependent secretion with a minimal effective frequency of 2–5 imp/sec and a maximal secretion at 15 imp/sec The present results, which show a noticeable secretion already at 0 5 imp/sec, seem to be at variance with those of Eccles & Wilson (1973) This can probably be attributed to the simultaneous activation of sympathetic vasoconstrictor fibres in their experiments Thus, the present results suggest that the secretory responses are activated even at a low rate of parasympathetic discharge This is further supported by the associated increases in tracer disappearance rate and local blood content, which indicate that the vascular and secretory responses in the nasal mucosa are activated simultaneously, in a a frequency response manner between 0 5–12 imp/sec

Quantitative data on the relationship between vascular effects in the nasal mucosa following

parasympathetic and sympathetic nerve activation respectively are not available In a previous study on the effects of sympathetic nerve activation (Anggård & Edwall, 1974) local application of a vasoconstrictor drug was found to result in a 80–90 % reduction in local blood content, when compared with the maximal response obtained by sympathetic stimulation

When the parasympathetic effects on local blood content are compared in a similar way with the maximal effects of sympathetic activation, as mimicked by local application of a vasoconstricting drug, the results indicate that local blood content and, indirectly, nasal patency is mainly influenced by changes in sympathetic tone However, the profound increases in tracer disappearance rate following parasympathetic stimulation at frequencies below 1 imp/sec suggest a strong parasympathetic influence on the exchange function, as compared to the sympathetic effects (Anggård & Edwall, 1974)

Morphological studies on human nasal mucosa have demonstrated that the nasal glands and blood vessels are richly innervated by cholinergic axons (Ishii, 1970, Cauna et al, 1972) The results of morphological studies on cat nasal mucosa are in agreement with these findings (Änggård & Densert, 1974) Eccles & Wilson (1973) demonstrated that nasal secretion owing stimulation of the Vidian nerve was ced by activation of cholinergic receptors hich were highly sensitive to atropine, complete blockade was seen after as little as 5 μg atropine/kg i v However, in the present study a slight secretion was occasionally observed at high frequency stimulation even after administration of atropine in amounts of 1 mg/kg This might indicate that the present technique is more sensitive to slight changes in nasal secretion or that the results of Eccles and Wilson were affected by the simultaneous activation of sympathetic fibres as discussed above

From the morphological studies showing a cholinergic innervation even around the blood vessels in the nasal mucosa it might be expected that the vascular responses to parasympathetic nerve stimulation would also be blocked by atropine However, the vascular respon reflected by tracer disappearance rate and blood content, could not be blocked by a even though nasal secretion was com abolished This suggests a functional di tiation between the mechanism behind th vation of secretory and vascular respo the nasal mucosa following parasymp stimulation

Even if stimulation of the parasymp nerves to an organ increases the regional flow, this is no proof of the existence of sp vasodilator fibres, as the vasodilatation equally well be induced by metabolites pr as a result of the increased tissue activity kow, 1955) Atropine resistant vasodila well known from studies on salivary gland has been attributed to the release of forming enzymes (Kallikrein) during act of glandular elements, with a subsequent f tion of vasodilating kinins (e g brad (cf Hilton & Lewis, 1955) This was conf by Gautvik (1970) who further sugges "all nerve fibres to the salivary glands cholinergic and that the neuroeffector tra sions vary in their atropine sensitivity" large numbers of glandular elements in th mucosa and the present findings that se and vascular responses are activated at th time might suggest a similar mechanism i nasal mucosa

Skinner & Webster (1968) suggested th atropine-resistant vasodilatation in the mandibular salivary gland was due to act of β adrenergic receptors This explanation unlikely in the nasal mucosa since in the p study a vasodilatation occurred even complete sympathectomy had been perf Furthermore, in a previous study no ev was found for the existence of β receptors ing the capacitance or exchange vessels nasal mucosa (Änggård & Edwall 197

In the present study the effects of para thetic nerve activation resembled those of arterial infusion of bradykinin which suggest that kinins are also responsible fo atropine-resistant vasodilatation in the

a However, the vasodilatation might also e to the release of vasoactive substances than bradykinin The negative results ed on stimulation or inhibition of the sis of prostaglandins indicate that the ilatory action is not due to the release of glandins

is the present results suggest that following ympathetic stimulation of the nasal mucosa ory and vascular responses are activated taneously and within the same frequency The local blood content and thus in- ly nasal patency, will only be moderately ed, while the tissue–blood exchange is in- ed even at a low parasympathetic discharge The secretory responses are due to activa- of cholinergic receptors, while the mechan- behind the activation of the vascular nses is still unknown

ZUSAMMENFASSUNG

Virkung der selektiven parasympathischen Nerven- erung auf die sekretorische Reaktion und auf das lare Austausch und Fassungsvermogen der Nasen- mhaut wurde an der Katze untersucht Dabei wur- le lokale Abnahme von [135]I und die Veranderungen ulsschlagzahl unter Verwendung von [131]I-markiertem nalbumin in der Nase gemessen Eine frequenz ab- ge Steigerung der Nasensekretion und des lokalen olumens trat bei einer Frequenz von 0,5–12 Im n/sec auf Gleichzeitig wurde eine Erhohung der ngquote beobachtet Diese Befunde deuten darauf lass die vaskularen und sekretorischen Reaktionen zeitig aktiviert werden Die sekretorischen Reak- n konnten durch Atropin gehemmt werden, nicht h die vaskularen Intraarterielle Bradykinin Infu n bewirkten eine Steigerung der Abklingquote ahn er nach parasympatischer Nervenstimulation wäh- Infusionen mit Arachidonsaure oder Indomethazin Wirkung zeigten Die Ergebnisse lassen erkennen, der postganglionare parasympathische Überträger erg ist, wohingegen die Vasodilatation von einem en durch Atropin oder Indomethazin nicht beein aren Mechanismus gesteuert wird

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ULTRASTRUCTURE OF THE EPITHELIUM IN ATROPHIC RHINITIS

Transmission Electron Microscopic Studies

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Abstract Biopsies taken from various locations in the nasal mucosa of patients with atrophic rhinitis were investigated in the transmission electron microscope Characteristic is a widespread metaplasia of the pseudostratified cylindrical epithelium to a keratinized squamous epithelium which corresponds very closely to thin skin On the basis of the ultrastructural study a hypothesis concerning the pathogenesis of atrophic rhinitis is put forward According to this theory an important feature in the development of some nasal symptoms is a decreased amount of secretory immunoglobulin A and consequently perhaps a reduced opsonising effect upon the bacteria in the secretion

While the presence of a horny squamous epithelium on the outer surface of the body is useful protecting the organism against trauma and ents dehydration, the presence of the same pe of epithelium in the respiratory tract is unphysiological and may be harmful in causing symptoms and disease It is known that chronic infectious reactions in the nasal mucosa can, to a certain degree, induce localized metaplasia of the ciliated epithelium to squamous epithelium (v Dishoeck & Majer, 1964) In addition, it is known that space creating operations in the nasal cavity can evoke an atrophic rhinitis in the squamous epithelium, though most atrophic rhinitis has a quite unknown etiology and pathogenesis However, Reichert & Hochstrasser (1971) recently demonstrated lack of a protease inhibitor in nasal secretion from patients with atrophic rhinitis This may be an important factor in the pathogenesis of the disease, though it is not known whether it is a primar secondary phenomenon

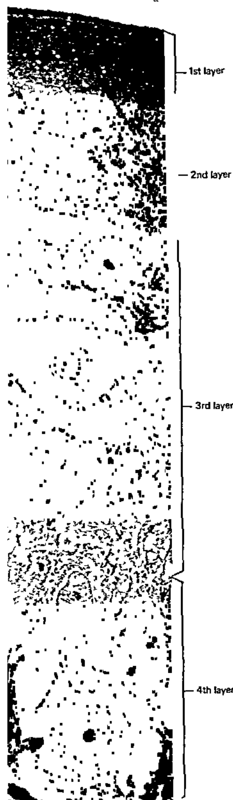
One assumption for an understandin causes of a disease is a knowledge of its logy In atrophic rhinitis Holopainen extensively investigated and reviewed microscopy, histochemistry and cytolog our knowledge no paper has been pu about the transmission electron micros this disease Recently we presented a p scanning electron microscopic studies atrophic nasal mucosa (Mygind et al. 19

MATERIAL AND METHODS

Six adult patients with pronounced rhinitis were examined For a longer p time they had all suffered from crust-f in the nose, and they were only treated b servative means

Biopsies of a size of 1½–3 mm were tak a small forceps without local anaesthesia were taken from the lower edge of the turbinate ½–1 cm behind the anterior edge as from other places in the nasal cavity

As glutaraldehyde renders the epithe herent to the forceps, the biopsies were from the forceps in 0.9% sodium ch immediately after transferred to a cold tion of glutaraldehyde, buffered with a sodium cacodylate (pH 7.4) After 24



fixation the biopsies were stored in the buffer. The specimens were post-fixed for 2 hours at 4° with 2 % osmium tetroxide, buffered with sodium cacodylate to pH 7.2–7 4 After dehydration in ethyl alcohol the blocks were embedded in Epon. Sections were cut in a Reichert ultra-microtome UM 2 with a Dupont diamond knife Semithin sections were analysed in anoptral contrast Ultrathin sections were double stained with lead citrate and uranyl acetate and transmission electron micrographs were obtained with a Zeiss EM 9A electron microscope.

RESULTS

A transitional epithelium as well as a horny squamous epithelium were seen in the biopsies At the biopsied location in the front of the inferior turbinate a transitional epithelium is often found in normal persons However, in a total of 100 biopsies from this site in normal persons and in patients with perennial rhinitis only four biopsies showed a keratinized epithelium (Mygind, 1974) Such an epithelium was found in 5 of 6 cases of atrophic rhinitis, and as a horny squamous epithelium therefore is characteristic of atrophic rhinitis, only this type of epithelium will be described (Fig 1)

The horny layer consists of 5–30 stratified keratinized cells with a thickness of 1 0–1 5 μm, i e a total thickness of the horny layer of 10–30 μm, on average The keratinized cells are anuclear without cytoplasmatic structures They lie close together with short interdigitating structures, which at the surface correspond to the short

Fig 1 Low power view of transmission electron micrograph of the nasal epithelium in atrophic rhinitis (×3 000)

1st layer (Stratum corneum), consisting in this specimen of 12 layers of keratinized, anuclear cells, each layer with a thickness of 1 0 μm.

2nd layer (Stratum granulosum) The cells are hori

nucleolus

4th layer (Stratum basale) The vertically arranged cells, containing large nuclei, are in contact with the very thin basement membrane



Fig. 2. Medium-power micrograph of the 1st and 2nd layer of the nasal epithelium *in patient* with atrophic rhinitis. The keratinized cells are anuclear, without cytoplasmatic structures. Short, interdigitating structures are seen, these are probably *remnants* of desmosomes. The cells of the 2nd layer have elongated nuclei [illegible] a fine chromatin pattern. The cytoplasm is [illegible] horizontal tonofibrils (*tf*), keratohyalin gran[illegible] few mitochondria (*m*) and scattered polyribosomes [illegible] (× 17 000)

Some short, round mitochondria (*m*) and scattered ribosomes (*r*) are seen Cytoplasm membrane (*cm*) with interdigitations (×30 000)

rils (*t*), continuing in the desmosomes (*arrow*)

scan-
1974)
ists of
ucleated cells, it becomes apparent that the
oned structures probably are remnants of
osomes The cells in this layer are spindle-
d with an elongated nucleus containing a
hromatin pattern. The cytoplasm is domi-
by several horizontal tonofibrils, some
ed kerato hyalin granules and a few mito-
ria At different levels in this layer cells
een with a more dark, condensed cyto-
a, representing a keratinizing process In

the third layer (Fig 3) the cells are irregularly polygonal with a large round nucleus containing a coarse chromatin pattern and 1–2 distinct nucleoli Also in these cells a dominant feature in the cytoplasma is the concentrically arranged tonofibrils which continue in the numerous desmosomes, which holds the cells together in collaboration with the folded cytoplasma membrane Some mostly short, round mitochondria and scattered polyribosomes are seen The fourth and lowest layer in the epithelium consist of vertically arranged cells, which in survey magnification have some resemblance to the basal

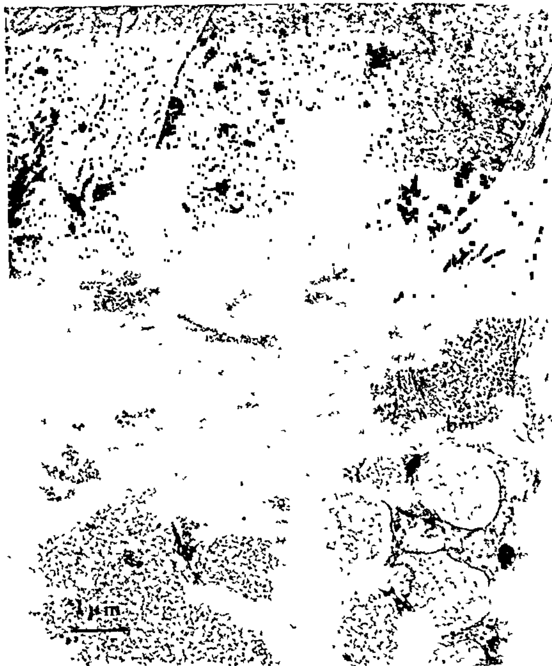


Fig 4 Transmission electron micrograph of the 4th layer of the nasal epithelium in patient with atrophic rhinitis The cells are less firmly attached to each other, but do contain some tonofibrils (*tf*) At the (*bm*) semidesmosomes (*sd*) are seen The membrane (*cm*) is thin and locally absent

the pseudostratified cylindrical epithelium However, even these cells are characteristic of squamous epithelium in containing a moderate number of tonofibrils, which at the basement membrane sometimes form semidesmosomes (Fig 4) Especially in the upper part of the cells typical desmosomes are seen, whereas the lower part of the cells are less firmly attached to cytoplasm also contains polyribosomes mitochondria than does the superficial the epithelium

The basement membrane is an membrane, which is so thin that at low fication it may appear interrupted

magnifications disclose a continuous ane The connective membrane too is thin ally absent

ids in lamina propria were rare and glandu-ts were very seldom observed When they resent, the epithelium of the upper part of t was of the same type as the surrounding asized epithelium

DISCUSSION

of the light microscopic observations of inen (1967) were confirmed in this ultra ral study, i e the presence of islands of ous epithelium, a considerable variation ckness and type of the epithelium, a decreased number of glands and gob-s and an absent or very thin connective membrane We agree with Holopainen e basement membrane is so thin that it e invisible in the light microscope, but the ructural study revealed a continuous, membrane

resemblance to skin was striking in the nized squamous epithelium with tonofibrils esmosomes even in the cells in apposition basement membrane and with a horny of the same order of size as that of thin (Menton & Eisen, 1971, Odland & Reed, This may imply that the atrophic epithe-has, to a certain degree, similar properties skin-epithelium, i e impermeability for The surface of the epithelium is there-not moistened from the tissue fluid, and e number of glands is highly reduced, ithelial surface necessarily becomes dry e metaplasia of the ciliated epithelium ls down into the glandular ducts, the expul of secretion from these may be impaired, cilia can assist in the extrusion of the secre-This may be a pathogenic factor in the ny of the glands

normal skin does not permit penetration munoglobulin A (IgA) and does not syn-e secretory piece (Lai A Fat et al , 1973), he case with the normal respiratory epithe .Therefore, the concentration of secretory IgA (two IgA molecules connected with one secretory piece molecule) in the atrophic mucosa may well be decreased in proportion to the area of metaplastic epithelium Secretory IgA has probably an opsonising effect on the bacteria in facilitating their phagocytosis (Doe, 1972) This matter creates the basis for a theory, which could explain some features in the pathogenesis of atrophic rhinitis

*In patients with this disease the bacteria nor*mally inhaled and filtered in the nose will not be caught by a continuous layer of mucus having a bactericidal effect and will not be transported to the rhinopharynx by the cilia, swallowed and destroyed by the gastric acid They remain *in loco* and multiply, possibly with the dead horny cells as nourishment The bacteria deliver factors chemotactic for neutrophilic leucocytes, which in large number penetrate the epithelium On account of the lacking opsonising effect (due to the decreased concentration of secretory IgA) the leucocytes cannot phagocytize and destroy the bacteria, but are themselves attacked by bacterial products The decreased leucocyte function brings about the constant production of chemotactic factors, which account for the continued penetration of neutrophils through the epithelium In addition, the multitude of dying neutrophilic leucocytes release proteolytic enzymes in the secretion These enzymes are not neutralized, due to lack of protease-inhibitor (Reichert & Hochstrasser, 1971) The free proteolytic enzymes in the secretion are probably harmful to the mucous membrane, as it is known that inhalation of proteolytic enzymes produces emphysema in animals (Johanson et al , 1973)

This theory can explain the following observation made by Holopainen (1) The large amount of bacteria in the nasal smear, (2) the large number of neutrophilic leucocytes in the secretion compared with the lamina propria, where the mononuclear cells dominate, (3) the lack of intracellular phagocytized bacteria in the neutrophils in the secretion, (4) the decomposed appearance of these leucocytes

The present ultrastructural study sheds no

light on the etiology of the disease, but shows that an important intermediate link in the pathogenesis is the factor or factors which influence tha basal cells in the epithelium to synthesize tonofibrils and desmosomes The fact is, that this feature causes the respiratory epithelium to metaplasize to squamous epithelium, since all cells are formed from the basal cells

It is possible that the bacteria in the secretion, i e Klebsiella ozenae (v Dishoeck & Majer, 1964), are able to induce formation of tonofibrils in the basal cells, or such may be the case with immunological mechanisms provoked by the bacteria Generally speaking, there is a total lack of information on the immunopathology of the nasal mucosa and on the content of antibodies in secretion in atrophic rhinitis

ZUSAMMENFASSUNG

Verschiedenen Stellen in der Nasenschleimhaut einiger Patienten mit atrophischer Rhinitis wurden Biopsien entnommen und im Transmissionsmikroskop untersucht Charakteristisch ist eine weit verbreitete Metaplasie des mehrreihigen Zylinderepithels in ein der dunnen Haut sehr ähnliches keratinisiertes Plattenepithel Aufgrund der ultrastrukturellen Untersuchung wird eine Hypothese um Pathogenese der atrophischen Rhinitis aufgestellt Da nach ist eine reduzierte Immunoglobulin A Sekretion ein wichtiger Faktor fur die Entwicklung einiger nasaler Symptome und vielleicht auch fur eine reduzierte opso niserende Wirkung auf die sich im Sekret befindlichen akterien

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SINOSCOPICAL BIOPSY IN MAXILLARY SINUSITIS

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·r 111 consecutive sinoscopic biopsies from the ı of the maxillary sinus in patients suffering from s were studied with regard to a number of histo- parameters and compared with the appearance mucosa during sinoscopy No characteristic histo- picture was found for either group of sinoscopic s but a statistically significant association was between on the one hand, thickening of the base membrane eosinophilia loss of epithelium and nd increased number of goblet cells and, on the the allergically dominated polypous and fibrous of sinusitis The reproducibility of the semiquanti histological grading and the representativity of the ·s were studied The individual biopsies were not to be representative with regard to all of the gical parameters It is concluded that the introduc f a flexible biopsy forceps with optic is necessary ther utilization of sinoscopic biopsy

ılogical studies of sinusitis have previously confined to mucosa removed operatively patients with chronic sinusitis and acute itis with complications Several differing ological classifications of sinusitis have been on the basis of the above

ute sinusitis is most often classified as rhal or purulent based on the amount of lar exudate, particularly polymorphonuclear ılocytes Chronic sinusitis is classified by asse (1923) and Andersen (1943) into (1) edematous or hyperplastic type, (2) an in tive or granulating type, (3) a fibrous type, (4) a mixed type

ie value of these classifications is limited by act that several histological types are fre tly found in the same specimen of mucosa hoff, 1954 Davidson, 1969, Köhn 1969) ldition Andersen (1943) and Schätzle (1963) frequently found disagreement between the clinical and histological types of sinusitis

Even though endoscopy of the maxillary sinus has been carried out in several centres since the beginning of this century, only a few studies have been published on mucosal biopsies removed by endoscopy Bauer (1958, 1960) stated that there was good agreement between the sinoscopic examination and the microscopical appearance of a biopsy from the maxillary sinus in cases of allergic and infectious conditions

We considered that it would be of interest to study how the changes, which are seen in biopsies taken from the mucous membrane of the maxillary sinus of patients suffering from sinusitis, correspond to the appearance of the mucosa during sinoscopy

In this respect we found that it would be necessary to investigate the validity of the histological grading and to what degree the single biopsy was representative of the mucosa as a whole.

MATERIAL AND METHODS

The material consists of 157 biopsies originating from 200 consecutive attempts at biopsy taken from patients suffering from clinically and sinoscopically diagnosed maxillary sinusitis In 43 cases either too little tissue had been removed or the tissue had suffered from too much trauma to be of use for histological examination

111 of the biopsies suitable for examination

Table I *Reliability of the histological examination*

I Two independent gradings of 100 biopsies
II The grading of two separate areas on each of 50 specimens from surgically removed mucosa

	Per cent consistency I	Per cent consistency II	Difference
Basement membrane thickening	81	58	$\chi^2=9.01$ ($p<0.005$)
Oedema		48	
Fibrosis	76	72	$\chi^2=0.28$ ($p>0.5$)
Lymphocytes		60	
Plasma cells		72	
Polymorphonuclear neutrophils		52	
Eosinophilia	76	54	$\chi^2=7.49$ ($p<0.01$)

originated from sinuses with one of the 4 sinoscopic pictures described below The remaining biopsies originated from sinuses with a mixed sinoscopic picture

The sinoscopic appearance of the mucosa in cases of sinusitis is divided into 4 types, as described by Illum & Jeppesen (1972), (1) an acute type with bright red, slightly swollen mucosa, (2) an oedematous type with pale, diffuse oedematous mucosa with a few dilatated vessels, (3) a polypous type, dominated by multiple, pale ›lyps, and (4) a fibrous type, with irregular ›tish solid areas between areas with oedema ›yps

The biopsies were removed from a limited area on the rear lateral wall of the maxillary sinus using a fine cup biopsy forceps (Illum & Jeppesen, 1972) The tissue was fixed in formalin and *cut into histological sections, which were stained* using hæmatoxylin eosin, van Giesons connective tissue stain and alcian green

The histological changes were registered by one of the authors without him having any clinical information whatsoever A semiquantitative grading was carried out ○–(+)–+ of some of the parameters loss of epithelium, squamous cell metaplasia, loss of cilia and goblet cells, and O–+–++–+++ of other parameters mucus in epithelial cells, basement membrane thickening oedema, fibrosis, lymphocytes, plasma cells, polymorphonuclear cytes and eosinophilia

The validity of the histological ... investigated by grading the basement me... thickening, fibrosis and eosinophilia ... consecutive biopsies from the ... months after the first grading and comp... results *The representativity of small are...* mucosa was evaluated by comparing the ... pendent gradings of two fortuitous area... sections from *maxillary sinus mucosa* ... operatively

RESULTS

Reproducibility of the histological grading

The *semiquantitative grading of the* ... meters studied could be satisfactorily rep... 2–3 months after the first evaluation D... ment was found in 19% following grad... basement membrane thickening ... ing figure for fibrosis and eosinophilia ... of the biopsies (Table I) A difference of ... than one grade was rare, however

Representativity of a biopsy

Table I also shows the results of the e... tion of two different areas of 50 specim... maxillary sinus mucosa removed at ope... expressed as the frequency ... of the parameters, which were graded ... A χ^2 test shows a significant differen... related gradings of the basement *thickening and eosinophilia*, while this ... case with fibrosis

The difference between the baseme... brane thickening and *eosinophilia in* ... of the mucosa thus cannot be expla... accuracies in the histological examin... this can be the case with *regard to* ... single biopsy cannot therefore be cons... representative with regard to the first ... meters Fibrosis appears to be mor... distributed throughout the mucosa

The frequency of suitable biopsies

Following 200 attempts at remov... suitable tissue was obtained in 157 case...

II Sinoscopical type of mucosal changes

	Acute type	Generalized oedema	Polypous type	Fibrous type	Significant difference
f epithelium					
+) – +	14/6	38/17	10/10	5/11	$p<0.05$
ous cell metaplasia					
+) – +	20 0	54/1	20 0	14/2	n s
t cells					
+) – +	16/4	42/13	10/10	12/4	n s
f ciliae					
+) – +	8/12	34/21	8/12	3/13	$p<0.05$
in epithelial cells					
+ / + + – + + +	12/8	28/27	9/11	10 6	n s
ent membrane thickening					
+ / + + – + + +	19/1/0	44/6/5	10 6/4	7/4/5	$p<0.001$
a					
/ + + – + + +	4/5/11	5/21/29	0/5/15	2/6 8	n s
is					
+ / + + – + + +	17/2/1	47/6/2	18/2/0	14 0/2	n s
hocytes					
+ / + + / + + +	8/12 0	21/30 4	4/15/1	10 6 0	n s
a cells					
+ / + + / + + +	7/11/2	8/45/2	1/14/5	6/9/1	$p<0.02$
orphonuclear leucocytes					
+ – + + / + + +	5/14/1	10 42/3	7/12/1	9/7/0	n s
ophilia					
+ + + / + + +	9/10/1	31/17/7	6/6/8	7/2/7	$p<0.025$
(111 biopsies)	20	55	20	16	

.e remaining patients there was either too tissue or it had been subjected to so much na that it was unsuitable for histological ex- ation The size of the suitable biopsies varied approx 1×1 to 4×6 mm and was most 2×3 mm

parison of histological and sinoscopical ges[1]

biopsies were distributed between the sino- c groups as shown in Table II The grading s detailed in this table on account of the tical analysis No characteristic histological re was found for each of the sinoscopic ps but a χ^2 test carried out for each of the idual histological parameters showed, how- some differences between the groups

significantly increased frequency of base- membrane thickening was found in the us and polypous types ($p<0.001$) This also ied to eosinophilia ($p<0.025$) An increased ency of loss of epithelium ($p<0.05$) and loss of cilia ($p<0.05$) was also seen in the fibrous type The polypous type was, in addition, domi- nated by plasmacellular infiltration ($p<0.02$) and goblet cells in the epithelium ($p<0.05$)

On the other hand no difference was found between the groups with regard to oedema and fibrosis, and there was no difference in the grading of the infiltration by lymphocytes and polymorphonuclear granulocytes

wish to thank H Engberg Pedersen M Sc Leo naceutical Products for performing the statistical sis

DISCUSSION

The four most important factors that can inter- fere with the evaluation of histological changes are (1) bias, (2) reproducibility of the registered findings, (3) the representativity of a biopsy of the whole pathological process, and (4) the tech nical quality of the biopsy

In the present study an attempt has been made to eliminate bias by carrying out the histological evaluation without having any knowledge of the clinical data Bias that occurs owing to the pres- ence or absence of other pathological changes could hardly be avoided

The reliability of the semiqua

tion used in the present study depends upon the reproducibility of the noted changes This source of error is rarely studied in pathological investigations, but Ringsted & Ferebee (1964) found for example with double blind evaluation of 1104 sections from Kweim's test that 8 % disagree when performed by one pathologist, while two pathologists were in disagreement on 21 % of the sections This agrees on the whole with our study, where a more detailed grading has been used, and where there was disagreement between one pathologist's evaluation when performed twice of 19–24 % of 100 biopsies, depending upon the parameters studied

The representativity of a single biopsy should actually be studied by independent evaluation of two biopsies from every sinus As it was often impossible to obtain more than one biopsy with the technique used, it was necessary to evaluate this parameter by examining two areas of an operatively removed specimen Single biopsies were found to be non representative with regard to thickening of the basement membrane and eosinophilia, while there was no demonstrable difference between places with regard to fibrosis This does not correspond to the impression one has of the irregularity of fibrosis during sinoscopy Kashiwado et al (1966) stated that a biopsy of mucosa was representative of , hole mucosa in 80 % of the cases

ne quality of the biopsy is an even more ›rtant factor with regard to the histological examination The average size and the frequency of successful biopsies are in agreement with Kashiwado et al (1966) Cup biopsies are, however, often somewhat compressed, particularly at the edges, which can result in the loss of oede matous tissue and the formation of fibrosis like artifacts This can be the reason why no difference was found in the occurrence of oedema and fibrosis in the various sinoscopic types of sinusitis

Thickening of the basement membrane and eosinophilia were found significantly more frequently in biopsies from maxillary sinuses with fibrosis and polypous sinoscopic pictures which are often seen in cases of prolonged allergy

Shambaugh (1931) and Weille (1930) a relationship between allergy and th the basement membrane

Loss of epithelium and cilia were f frequently with the fibrous type whi in agreement with Shambaugh (1931), a pronounced association betwee membrane thickening and loss of e The increased occurrence of goblet c polypous type is in agreement wi (1963), who found an increased numbe cells in the epithelium in allergic cor

No significant difference was fou occurrence of polymorphonuclear g in the 4 groups This can possibly n the non uniform distribution of these submucosa, but also that they have n with inflammatory activity than to th of the inflammation (Lucas, 1952) (1943) found that polymorphonucl often absent even in cases of puruler

There was no difference either sinoscopic groups with regard to the of lymphocytes, while plasma cells most frequently in the polypous type

CONCLUSION

There was thus only found a certain agreement between the macroscopical scopical appearance of the maxillar cases of sinusitis The most important possible causes of this is probably th able variation in the histological cha one site to another in the mucosa with the fact that it is not possible biopsies under direct vision Endoscop will presumably be of most import regard to the study of localized proce maxillary sinuses and the introduction ible biopsy forceps with optic is a p for further progress

ZUSAMMENFASSUNG

111 konsekutive sinoskopische Biopsien der k schleimhaut bei Sinuitis wurden blind un eine Reihe von histologischen Parametern

ırden mit dem Bild der Schleimhaut bei Sinoskopie ıen

vurde kein charakteristisches histologisches Bild Gruppe der sinoskopischen Bilder gefunden, ·n aber ein statistisch signifikanter Zusammenhang ·n einerseits Basalmembranenverdickung Eosino-Epithel- und Zilienverlust sowie einer vermehrten l Becherzellen und andererseits den von allergischen dominierten polyposen und fibrosen Sinuitis-n

Reproduzierbarkeit der semiquantitativen, histo-en Gradierung und die Reprasentativitat der : n wurden untersucht Die einzelne Biopsie war bei ıistologischen Parametern nicht reprasentativ

ussfolgerung Das Benutzen einer beweglichen ·zange mit Optik ist fur ein weiteres Ausnutzen der :opischen Biopsie erforderlich

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A METHOD FOR STANDARDIZED STUDIES OF MUCOCILIARY ACTIVITY

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(Received November 17, 1973)

Abstract A method for observing ciliary activity of mucous membrane is described and tested Ordinary laboratory equipment is used Variations of surface light reflections due to secretional wave movements are recorded under various conditions The temperature and humidity parameters can be controlled The method is standard ized with regard to the following variable factors

(1) intensity of light source, (2) optical and electrical magnification, (3) extent of area observed, and (4) re cording speed Rheological aspects of the mucociliary system will be discussed further in subsequent papers together with temperature and humidity effects

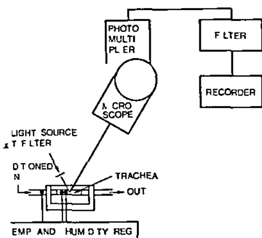
There are two main approaches to the study of the mucociliary function of the upper respiratory tract One is qualitative observation of the transportation rate of small particles (Sharpey, 1835, ill, 1928, Hilding 1932, Messerklinger, 1951, hamn, 1956, Ewert, 1965) or quantitative ervation of the removal of a known amount of deposited or inhaled particles, i e clearance estimations (Baetjer & Bates, 1966, Baetjer, 1967, Rylander, 1968, Quinlan et al, 1969) The other is to record the beat frequency of the cilia or, more correctly, the frequency of mucous waves brought about by the beating cilia The latter approach was used as early as 1884 when Martius estimated the ciliary beat frequency with a stroboscope The stroboscopic technique has been further developed by Gray (1931) Ballenger & Orr (1963) and by Andersen (1971) Photographic techniques were introduced by Gray (1931), Lucas (1931), Proetz (1932), Frenckner & Richtner (1939) and by Dalhamn (1956). M using a photocell with a focusing scr TV screen display were published by D & Rylander (1962) Håkansson & To (1965), Guillerm et al (1965) and by T (1967) Chevance & Lennon (1970) desc microphoto–oscillographic method in t photomultiplier

Stroboscopic methods do not permit frequency changes to be followed and cine graphic methods are time-consuming and e sive for routine experiments where im results are wanted The modern methods photocells or photomultipliers seem the most suitable for this type of experiment

Respiratory tract mucosa is preferred f perimental tests regarding the effect of ch substances and air pollution materials o function (Negus, 1934, Proetz, 1953 Da 1956, Krueger & Smith 1958, Dahlh 1972) The problem is, however that se methods and types of equipment are m it is impossible to compare in detail the from different research groups In our o there is an urgent need for a relativ method for ciliary function studies, wh be easily reproduced in most experimen clinical laboratories

The aim of the present investigation design and test a method based on experimental experiences (Håkansson & malm, 1965, 1966 *a* and *b*, 1967, 196 et al, 1972) and meeting the following ments

This investigation has been supported by grants from the Swedish Medical Research Council (Project number B73-14X-3897-01)



Block dıagram of the experımental equ pment (see

ıstantaneous recordıng of wave movements ı the secretıon layer ın experıments of long uratıon
ensıtıvıty to rapıd frequency changes
Jncomplıcated analysıs of the recordıngs
ımultaneous recordıng and regulatıon of the mperature and humıdıty parameters
Jse of routıne laboratory apparatus

METHOD

experımental arrangement consısts of (1) a t source (2) an experımental chamber for ıtro specımens (3) a bınocular mıcroscope ı photomultıplıer (5) a frequency fılter and an ınk wrıter as seen ın the block dıagram ıg 1 The laboratory set up ıs shown ın 2

The lıght source ıs a fıbre optıc system rz) clınıcally used for endoscopy The appa ıs has fıve ordınary grades of lıght ıntensıty ı a relatıon 1 2 3 5 4 2 6 5 There ıs no asurable heat grad ent near the mucous mem ne between the m nımum and maxımum nsıties The spectrum of the lıght source asured ın the mıcroscope ocular ıs 400–850

ʌ perspex experımental chamber accordıng to kansson & Toremalm (1965) ıs used It ıs ıble walled for ınsulatıon purposes and suppled wıth known amounts of temperated and humıdıfıed aır from a thermostat The condıtıoned aır ıs blown through the trachea where the temperature ıs measured by thermocouples The present experıments have been performed at temperatures between 20° and 37 C and a rela tıve humıdıty above 90 %

The bınocular stereomıcroscope or operatıon mıcroscope (Zeıss) ıs modıfıed so that ıt can be manoeuvred ın all dırectıons Thıs makes ıt easy to focus a suıtable spot on the mucous membrane preparatıon Two specıal oculars (Zeıss 25 ×) are used One has a specıal grıd for exact ıdentıfıcatıon of object posıtıon A dıaphragm ıs mounted ın the other ocular Thıs consısts of a dısc wıth a central openıng of 0 1 mm dıameter Several dıscs wıth other apertures have also been tested The hole must be posıtıoned exactly ın the centre of the optıcal axıs The edge of the hole ıs bevelled at that sıde of the dısc whıch poınts away from the observer s eye ın order to avoıd local reflectıons whıch may ınter fere wıth the orıgınal ones The area to be observed ıs fırst ıdentıfıed and focused ın the centre of the grıd Then the grıd ocular ıs removed and the dıaphragm ocular ıs put ın place On top of thıs second ocular a photomultıplıer (EMI 9524 B) ıs mounted It ıs fed by 1 000 V d c vıa a power supply (Oltronıx A 2 5 K 10 HR) A total optıcal magnıfıcatıon of 100 × ıs used

A frequency clıppıng fılter (Krone Hıte type 3550) ıs used ın order to exclude occasıonal dısturbıng frequencıes e g from the electrıc maıns Frequencıes below 3 Hz and above 30 Hz are elımınated

A dırect wrıtıng recorder (Elema Mıngograph 34) ıs used for recordıng of the lıght reflectıon varıatıons It has a frequency range of 0 to 700 Hz The paper speed has been eıther 25 or 100 mm/sec Normally a 1 mınute sectıon of the record ıs analysed The noıse level of the amplı fıcatıon system was measured to 200 μV The sıgnals obtaıned are 24 dB above thıs level

For the actual experıments the rabbıt trachea ıs mounted ın the experımental chamber ımmedıately after the anımal has been kılled by a blow

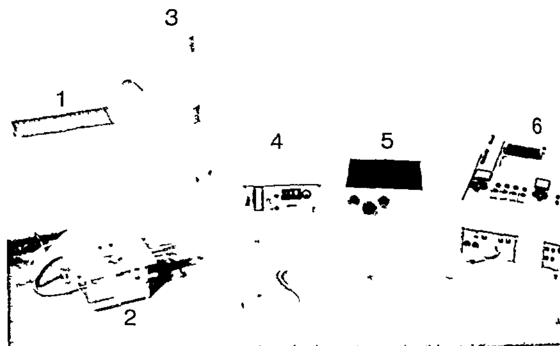


Fig 2 Details of the experimental equ pment *1* l ght source ? experimental chamber *3* microscope and photomult pl er *4* magnifier *5* frequency f... writer

on the head in order to avoid the influence of anaesthetic drugs Rabbits with a weight of 1 5–2 kg have been used

All experiments have been made on a non rating table in an electrically shielded room

RESULTS

The present equipment for indirect observation of the ciliary activity of respiratory epithelium includes different optical electrical and mechanical details The capacity of each is basically tested by the manufacturers When combined into a system they need to be trimmed in order to give maximum efficiency and a minimum of undesired disturbances Some parameters of the experimental situation are kept constant e g the choice of experimental animal preparation technique environmental temperature and humidity Other parameters have been experimentally tested i e (1) intensity of the light source (2) magnification of the microscope (3) the extent of the supervised area regulated by various diaphragms in front of the photomul... magnification of the photomultipl... and (5) the speed of the recorder

The experiments with increasing li... sity are demonstrated in Fig 3 Five d... degrees of intensity have been tested ... in the figure the light intensities ... grades 3 4 and 5 are adequate s... cordings can be easily analysed Grade ... as a routine light intensity

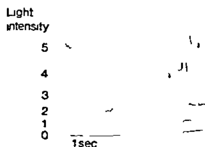
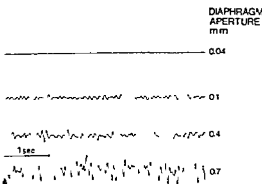


Fig 3 Light intensity and ampl tude varia... cord ngs are made from the same tracheal ... d fferent l ght intensit es Temp 27 6 C R... m dity >90 %. Diaphragm diameter 0 1 ... speed 100 mm sec



f The relationship between supervised area (regul-
by different diaphragm apertures in front of one
r) and the recorded secretion wave patterns Temp
C Relative humidity >90% Light intensity =3
rding speed 100 mm/sec.

ifferent degrees of magnification have been
d and a total optical magnification of 100×
proved to be suitable for ciliary function
ies as the wave pattern can easily be iden-
l

is necessary to focus an area wide enough
epresent the wave movements initiated by
oup of coordinated ciliary cells and small
igh to exclude too many interchanging wave
ements The choice of diaphragm is there-
of significant importance In order to find
itable observation area four different discs
used with round central openings of 0 04,
0 4 and 0 7 mm diameter respectively The
lts are demonstrated in Fig 4 If the opening
o small no light reflection changes can be
tered (Fig 4 upper) If the opening is too
, however, too many different waves are
which complicates the interpretation of the
rdings (Fig 4 lower) An opening diameter
1 mm gives an uncomplicated recording

e I

ure ter	Square area (μm^2)	Ciliary cells (approx)	Cilia (approx)
	22 690	600	145 000
,	3 320	90	21 000
	380	10	2 400
	79	2–3	500–700

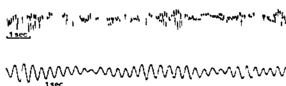


Fig 5 Original record from the rabbit trachea at a temp of 33 2°C and relative humidity >90% Light intensity =3 Diaphragm diameter 0 1 mm Paper speed for routine follow up = 25 mm/sec (*upper*) Paper speed for analysis and calculation = 100 mm/sec (*lower*)

from which the frequency can easily be calculated This means that a square area of about 380 μm^2 is analysed An area of this dimension covers approximately 10 ciliary cells comprising about 2 400 cilia (see Table I) With the use of this "ideal" observation area, rhythmic amplitude and frequency changes are seen (Fig 5)

The sensitivity curve of the photomultiplier is well within the range of the frequency spectrum of the light source The power supply of the photomultiplier was 1000 V during the entire experimental series

The standard paper speed for all the experiments is 25 mm/sec (Fig 5 upper) To facilitate the determination of frequency, a speed of 100 mm/sec is recommended (Fig 5 lower)

Fig 6 shows the effect on the record, when the light source is switched off This maneouvre is used for calibration purposes

DISCUSSION

The study of the mucociliary system of the respiratory tract is of growing interest in view of increasing local and personal air pollution and the effect of pharmaca, e g mucolytic drugs, which are applied directly to the mucous membranes The interest of such studies has led to the development of numerous methods and



Fig 6 Test to check for mechanical, optical and electrical disturbances Temp 38 0°C Relative humidity >90% Light intensity before and after test = 3 Recording speed 25 mm/sec

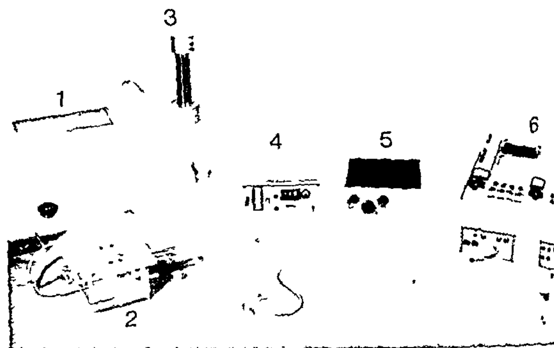


Fig 2 Details of the experimental equipment *1* l ght source *2* experimental chamber *3* m croscope and photomultiplier *4* magnifier *5* frequency [illegible] writer

on the head in order to avoid the influence of anaesthetic drugs Rabbits with a weight of 1 5–2 kg have been used

All experiments have been made on a non-[illegible]brating table in an electrically shielded room

RESULTS

The present equipment for indirect observation of the ciliary activity of respiratory epithelium includes different optical electrical and mechanical details The capacity of each is basically tested by the manufacturers When combined into a system they need to be trimmed in order to give maximum efficiency and a minimum of undesired disturbances Some parameters of the experimental situation are kept constant e g the choice of experimental animal preparation technique, environmental temperature and humidity Other parameters have been experimentally tested, i e (1) intensity of the light source (2) magnification of the microscope (3) the extent of the supervised area regulated by various diaphragms in front of the photomul[illegible] magnification of the photomultiplier [illegible] and (5) the speed of the recorder

The experiments with increasing li[illegible]sity are demonstrated in Fig 3 Five [illegible] degrees of intensity have been tested [illegible] in the figure the light intensities ind[illegible] grades 3, 4 and 5 are adequate si[illegible]cordings can be easily analysed Grade [illegible] as a routine light intensity



Fig 3 Light intensity and ampl tude vari[illegible] cordings are made from the same trache[illegible] different l ght intensities Temp 27 6 C R[illegible] midity >90 % Diaphragm diameter 0 1 [illegible] speed 100 mm/sec

fläche werden unter verschiedenen Bedingungen triert Temperatur und Feuchtigkeit konnen kon-

/ankungen diskutiert werden

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FOLDING MECHANISM OF THE HUMAN LARYNX

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(Received November 5 1973)

Abstract The mechanism of the human larynx often regarded as sphincteric more nearly represents graduated folding or plication Taking the end of normal expiration as the reference condition folding decreases with inspiration and increases successively with reserve expiration phonation effort closure and swallow closure

When fully closed the human larynx can successfully resist the greatest efforts of the body to blow it open The mechanism of this hermetic closure puzzled many early observers Dodart (1700) had called the glottis a rectilinear sphincter capable of narrowing without shortening Negus (1929) proposed that the striated muscles closing the larynx were components of a sphinc- , phylogenetically descended from a circular ncter closing the entrance to the primitive hea Doubt about this derivation arose from e fact that in Protopterus, the South African lung fish ostensibly a representative of the primitive state, the sphincter consists of smooth muscle (Goeppert, 1937) Certainly, in the human larynx, the anatomical arrangement of the striated muscles is not that of an ordinary sphincter A different and persuasive explanation incriminating check valve action emerged about a hundred years ago from the studies of Wyllie (1866), Lauder-Brunton & Cash (1883), and others, but when X ray investigation was brought to bear on the problem, no sign of a check valve could be detected (Lindsay, 1940) At present the true nature of the closing mechanism remains ill defined Pressman (1944) proposed a combined sphincteric check valve mec but this is opposed by an increasing a- X-ray evidence (Fink, 1956, Ardran & 1967) and is either in abeyance or re cognition for want of a clear alternative (1972)

This work was supported by USPHS Research Center Grant GM 15991

Interestingly enough the elements for interpretation have long been at hand T like arrangement of soft tissues in the was pointed out by Bertin in 1745 and cussed by the anatomist Henle at least as 1866 (Henle, 1866) The Basle Nom tomica (His, 1895) named three such fol vocalis, plica vestibularis, plica arye Several developments make it reaso propose that plication—the formation c tuation of folds—rather than sphincter is the mechanism of the main chang configuration of the human larynx

Objections to the sphincteric theor

A sphincter as defined in dictionaries (English Dictionary, Larousse) signifies or annulus of muscle that normally constriction of a bodily passage In the muscle sphincters of the esophagus and fibers most intimately related to the form a sheath to the canal and have no attachments In the smooth muscle sp of the stomach, the bladder and the the arrangement of fibers is circular these descriptions fits the morpholo intrinsic muscles in the human laryn

unctionally, the orifice guarded by a sphinc- opens briefly when material is pushed through passage during relaxation of the sphincter the mammalian larynx it is closure of the sage that is momentary, and most of the e the passage is open Moreover the open e in the human can be at least partly main- ed without a dilator muscle, as is seen during natory closure of the glottis, when abduction he arytenoid cartilages is inoperative yet the ibule remains open

n sum, irrespective of phylogenetic origins, striated musculature of the larynx in man onger has the morphological or physiological ibutes of a true sphincter, and produces a ncteric closure only in quite a loose sense the term I now attempt to show that folding nearly describes the sequence of events

ative features of the laryngeal mechanism

se may be considered under four headings iration, phonation, effort closure (coughing, k effort, defecation, and the like) and swallow ure, representing the four major patterns of avior into which the human larynx is organ- At the outset it is appropriate to stress Kotby & Haugen (1970*a*) that the laryngeal hanism is highly complex and that individual ations in the role of particular components to be expected

iration

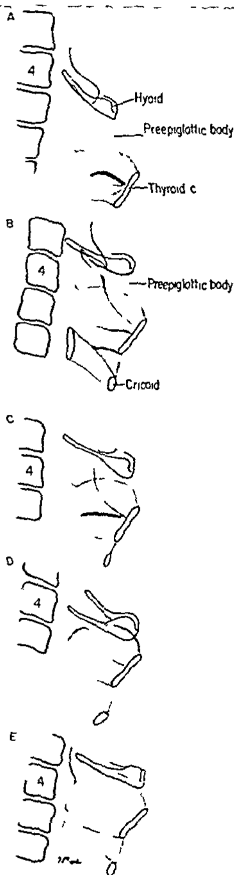
respiratory cycle vertical movements take in the diaphragm and in the train of carti- comprising the trachea and larynx Inspi- n leads to descent of the trachea and stretch of the laryngeal soft tissues near the hyoid The vestibular folds and aryepiglottic and the preepiglottic body become elon- l vertically and thinner transversely (Figs and 2A) in association with increased sepa- n of the hyoid bone from the thyroid and noid cartilages Viewed as a whole these ges may be said to represent a process of lding The arytenoids, constrained by the -arytenoid articulation to travel downward as well as outward, eventually rock backward (Fink et al, 1956, Ardran & Kemp, 1967), apparently obedient to the tension in the folds The effect of this is to flatten the vocal folds against the side wall, a further unfolding Expiratory ascent reverses the displacements (Figs 1B and 2B) that is to say it produces partial folding aided by recoil of the elastic tissue stretched in the descent (Fink, 1974*b*)

The role of the crico-arytenoid muscles in this cycle has been much debated Current evidence seems to favor the view that in man the muscles stabilize the upright posture of the arytenoids rather than directly adduct or abduct these carti lages (Kotby & Haugen, 1970*b*) Accordingly at the end of normal expiration the arrangement of the laryngeal structures in part reflects an equilibrium between passive forces and may conveniently be taken as the reference condition Inspiration then represents an active decrease in laryngeal plication, reserve expiration an active increase, linked respectively to the active augmentation and the active reduction of lung volume

In general, the larynx and the lungs are con stantly varying in shape and size with respiration, their variations being coupled neuronally through the respiratory center and mechanically through the trachea Part of the significance of plicatory coupling evidently resides in the fact that it relates the changes in volume and air flow resistance of the laryngeal air space to the changes in volume and flow resistance of the pulmonary air space

Phonation

Phonatory adduction of the arytenoid cartilages draws the posterior attachment of the vocal folds to the midline The adduction of the arytenoids is facilitated by the presence of the pyriform fossa but the associated excursion of the vocal folds is constrained by the thyroid cartilage Molding of the folds by contraction of the vocalis muscle is facilitated by the concomitant partial closure of the crico-thyroid angle or "visor", which in effect makes vocal fold tissue available for redistribution toward the midline The result



may be reasonably described as increas tion of the vocal fold

Folding of the vestibular folds sca creases in phonation, these folds are medially passively with the arytenoids a same time pulled slightly upward by tension in the aryepiglottic folds which tends to enlarge the ventricle (F and provides space for a large ampl vibration of the vocal folds (Muller, 18 1962) Additional laryngeal folding d at the level of the preepiglottic body (e tubercle, see below) with many vocal t mants as a result of vertical movements hyoid bone and larynx (Perkell, 1969).

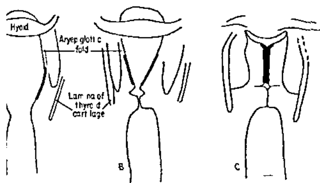
Effort closure

In efforts such as coughing defecation, or a heavy weight, a temporary hermetic of the larynx develops in which plicatio vestibular and aryepiglottic folds is a that of the vocal folds

The adduction and plication of the vo fold is thought to be effected by the thy noid muscle, which has fibers running upward and backward, and draws the v fold not only medially but also downwar obliterates the ventricle and apposes the ular folds to the adducted vocal folds.

The aryepiglottic fold, containing the end of the cuneiform cartilage is also during effort closure It folds along the lateral margin of the cuneiform (Figs 1 2C), which was called the point flexio aryepiglottic fold by Stuart (1892), so the

Fig 1 Idealized sketches based on X-ray illustrating the folding (plicative) nature of laryngeal mechanics Lateral view (*A*) In Aryepiglottic, vestibular and vocal folds and pre body elongated and flattened (*B*) Expira glottic, vestibular, and vocal folds and preep somewhat shortened (*C*) *Phonation*— Plication of vocal fold partial plication of p body Vestibular fold separated from the opening the ventricle (D) *Effort closure*— vocal, vestibular, and aryepiglottic folds of the preepiglottic body (E) *Swa* Marked plication of preepiglottic body epiglottic cartilage at the base of the epiglottis



2 Idealized sketches based on tracings of antero-rior tomograms of the human larynx The position e cuneiform cartilages (black bars) is inferred from l eye observations with a Macintosh laryngoscope *Deep inspiration*—Aryepiglottic folds elongated iarrow, vocal and vestibular folds flattened against id cartilage lamina (B) *Deep expiration*—Aryepiglottic folds shorter, vestibular and vocal folds thicker representing the reference condition of partial plication (C) *Effort closure*—Plication of aryepiglottic, vestibular and vocal folds. Cuneiform cartilages in apposition Ventricle almost obliterated The apparent angle of divergence of the thyroid cartilage laminae (angle of flare) decreases slightly from A to C

iform moves medially to the midline while rest of the aryepiglottic fold is directed ally, applied to the back of the epiglottic lage Since the aryepiglottic fold indirectly s to link the thyroid cartilage to the hyoid , its folding or plication is probably facili-l by approximation of the thyroid to the d, much as plication of the vocal folds is ted by approximation of the thyroid to the id Approximation of the thyroid and hyoid fact an integral part of effort closure of the ix and also causes the tubercle of the epi-ic cartilage to bulge backward a little (Czer-, 1861, Fink, 1956) and contact the plicated bular and aryepiglottic folds The midline osition of the cuneiforms involves strain of issue joining the cuneiform to the arytenoid lage, and the elasticity of this tissue assures on release of the strain the cuneiform auto-cally returns to its lateral position In this the cuneiform–arytenoid zone of strain is her of the many springs that secure the open iguration of the human larynx (Fink, 1973*a*)

low closure

types of swallow closure of the larynx have identified (Ramsey et al, 1955), one used rinking a long draught without pause by ring a drink down one s throat", during which the larynx remains low and the epiglottis upright, the other involving elevation of the larynx, forward propulsion of the hyoid, and retroversion of the epiglottis In both, approximation of the larynx and hyoid distorts the pre-epiglottic body, causing the lower part of the epiglottic cartilage to bulge backward as noted above under effort closure In addition, in the complete swallowing act the epiglottis often folds backward and downward and undergoes a final plication that caps the other plications already present lower in the larynx (Fig 1E)

Rationale of plication

Several features of the human laryngeal mechanism are easier to understand as plication than as sphincteric action

That the gradations of closure in expiration, phonation, effort closure, swallow closure, are better described as graduated folding than as graduated sphincteric closure emerges from consideration of the closure of swallowing and its tier of four foldings, two antero-posterior ones, the vocal and vestibular, surmounted by a T folding—the aryepiglottic—and a transverse folding, the epiglottic Crossing of plications at the T clearly increases the security of closure, but eludes interpretation in a sphincteric scheme Indeed the two upper stages are scarcely sphinc-

teric even in a broad sense, but need no qualification as foldings, accumulated successively in effort and in swallow closures

Sphincteric closure encompasses only the actions of the small, intrinsic muscles of the larynx, but folding takes into account the effects both of extrinsic and intrinsic muscles and unifies them into a single mechanically coordinated system For example, the elevation of the larynx during swallowing is understandable in part as a vertical shortening necessary for completion of the four-tier plication, as a condition of complete sphincteric closure the elevation lacks rationale Similarly, the vertical rhythm of the trachea during respiration can mechanically couple to folding and unfolding of the laryngeal folds, but not to sphincteric rhythm of the folds Finally, a newly described sagittal pliability in the larynx (Fink, 1973*b*) essentially a slight folding and unfolding of the thyroid cartilage at the isthmus, attributable to extrinsic muscle action is readily understood as integrated with the vertical movements of the larynx but has no apparent connection with sphincteric action

Last, but not least, the term "plication" possesses a useful semantic attribute Where disorders of sphincteric closure tend to be thought of as disorders of neurological control, disorders f plication suggest two kinds of possibilities, turbances of control and disturbances of a urely mechanical order, a distinction that is of potential clinical significance

ZUSAMMENFASSUNG

Der Mechanismus des menschlichen Kehlkopfs der oft als sphinkterisch betrachtet wird stellt eher einen differenzierten Faltungsprozess dar Wenn man das Ende der normalen Ausatmung als Bezugsbedingung ansieht so findet man dass die Faltung mit der Einatmung ab nimmt und bei reservierter Ausatmung Phonation Kehlkopfschluss bei Anstrengung und Kehlkopfschluss beim Schlucken stufenweise zunimmt

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MICRODYNAMICS IN VOCAL FOLD VIBRATION

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act The question what causes damage to the vocal in habitual faulty use of the vocal organs, is an- d in part by the morphological characteristics of the folds Experiments with a large stress exerted on the ligaments do not clearly support the hypothesis rupture of collagen fibres may occur in vocal strain

larynx when functioning in phonation can xposed to considerable stress Two forces ibute to this stress the air stream and the le contractions The total mechanical power e air stream is estimated as being approxi- ly ½ watt (Salmon, 1964) Only a small part 05 to 0 5 %) of it is transformed into acous- nergy The remaining energy is transferred at to the air stream and the vocal folds question arises, whether the larynx is able dure the influence of the transmitted energy, a may perhaps be traumatizing Are the acting forces of the laryngeal muscles big gh to cause trauma to the vocal fold tissues? aim of the present article is to survey this ion, paying special attention to the con- ve tissue

e transfer of energy from the air stream to ocal fold can be illustrated easily by the wing experiment A transparent finger of a ic surgical glove is filled with water in which pieces of paper are suspended This "vocal is pressed against a hole in a transparent ic plate When the pressure is adequate an ream directed through the hole will result rations of the model and a clear voice can ard At the same time strong rotatory and turbulent movements of the pieces of the paper *inside* the model can be observed It is conceivable that such movements would occur in the actual vocal fold if it were composed homogeneous, as is the water in our experiment In the vocal folds however the inner movements are considerably damped, mainly by the connective tissue which makes up about half of the contents of a vocal fold In the connective tissue collagen and elastic fibres form a matrix in a very viscous solution The special arrangement of these fibres modifies and restrains the tissue movements inside the vocal folds Certain parts of the connective tissue, e g the middle part of the vocal ligament, are more exposed to the effects of whirling vibration energy than are other parts If the quantity of absorbed energy is too large, traumatization of the connective tissue may occur

We have asked ourselves the following questions How strong is the resistance of the connective tissue of the vocal folds to mechanical trauma? Of what magnitude are the forces acting on the ligaments? To what extent is the anatomical construction of the vocal fold related with these forces?

Connective tissue in general is composed of connective tissue cells and of extracellular fibres (Bloom & Fawcett, 1968) They are embedded in an amorphous substance containing tissue fluid There are at least three types of extracellular fibre collagenous, and more and less elastic fibres, in parallel and in reticular formation Recent evidence indicates that collagenous and

reticular fibres may simply be different morphological expressions of the same fibrous protein (Gould, 1968) The chemical composition and physical properties are relatively well known It is generally accepted that the primary function of collagen fibres is a mechanical one, that is, to resist tension along their axis (Harkness, 1968) The orientation of the fibrils would be in response to mechanical stress and to cellular influences such as geometric necessity (Holm, 1963) Collagen is about as strong as the strongest known animal or plant fibres (Morgan, 1963) Its extensibility is less than 5% in the normal range of tension, when the fibres are parallel, but up to about 30% in the case of fibres forming feltworks Tendons contain about 30% of collagen on a wet weight basis The ultimate strength of mammalian tendon subjected to longitudinal tension is from 5 to 10 kg/mm² or, in terms of collagen, 15 to 30 kg/mm² According to Harkness it seems likely that the true strength of the collagen is that found in the smallest fibres, the larger ones consisting of aggregates of smaller ones, which are not ideally arranged and thus do not share the load equally Consequently, some can be broken before others, with a net reduction in strength With increasing stress the tissue becomes increasingly less extensible The early part of this change in mechanical properties is associated with a visible straightening of initially wavy collagen bundles (Harkness, 1968) The tensile strength rises during growth to a plateau and later falls in old age (Rollhäuser, 1950, Stucke, 1950, Yamada, 1964) Elastin differs in some respects from collagen with which Harkness found that elastin in its pure form can be extended to twice its resting length In elastic fibres breaking occurs when they are stretched to about 150% of their original length This requires a force of only 0 2–0 3 kg/mm²

Shrinkage by heat is another property of interest for a hypothesis on vocal nodules The shrinkage temperature varies with the different collagens The low values of 55° to 61°C were obtained from a 34-week-old foetus and the highest values, 63° to 67°C, from a 61-year old man (in fascia lata) (Brown et al, 19[illegible] shrinkage temperature in water for [illegible] collagens is 24° to 29°C above the [illegible] temperature (Gould, 1968)

Arrangements against Traumatizing F[illegible] Caused by Internal Friction (He[illegible]

Lymphatic and blood circulation can be [illegible] to prevent the excessive heat prod[illegible] vocal folds Our knowledge of this ef[illegible] ferent voice habits is, however, very [illegible] Basic studies are still lacking

In the ligaments and tendons the elas[illegible] form a network The spaces betw[illegible] fibres are filled with a delicated feltwork [illegible] lagenous fibres and a few fibroblasts [illegible] interfibrillar spaces there is a collo[illegible] consisting of polysaccharides and [illegible] acid which, by binding varying [illegible] water, can change its volume If it were [illegible] the interfibrillar material, the tissue w[illegible] extensible until the fibres were fully s[illegible] Now the interfibrillar mass offers [illegible] the deformation of the interfibrillar spa[illegible] ing to a decrease of volume, the ma[illegible] spaces between the fibrous partitions [illegible] pressed The polysaccharides being [illegible] paratively small molecular weight, are [illegible] importance for the water binding [illegible]

Hyaluronic acid, however, can [illegible] amount of water up to 2 000 times its [illegible] when it is in hydrodynamic equilibri[illegible] hyaluronic acid molecule is a long [illegible] thread like molecule which, when not [illegible] to an external force, will curl to a [illegible] It can be distended to an almost inf[illegible] but when left to itself will immediate[illegible] into a ball This is due to the mutual [illegible] of the water ions with which the [illegible] coil are clad For this reason hyaluronic [illegible] the ideal physiological greasing subst[illegible] fill any space between moving surfa[illegible] impeding the movements by friction [illegible] been demonstrated for the articular [illegible] of joints and for the umbilical cord [illegible] fibrous lamellar surfaces are covered [illegible] monomolecular layer of hyaluronic [illegible]

ıanism ıs lıkely to be met ın any sıtuatıon e one layer ıs slıdıng loosely over the other, the gut, the skın, and also ın the subepıthe- ıyer of the vocal folds and vocal lıgaments ere are several phenomena ın connectıon the human voıce whıch may have to do wıth chemıcal and physıcal propertıes

Every voıce needs a perıod of warmıng up 'tercıse before ıt can perform ıts full range ch and volume If the warmıng up perıod ıs cted, hıgh ıntensıty and pıtch are only ar- at by straınıng, and damage to the vocal ı may result Warmıng up perıod, e g ın , ıs often explaıned ın terms of adaptatıon e blood cırculatıon by work Local blood l *reflexes are, however, so quick that a* y state would be reached sooner Thus they ot account for thıs phenomenon We are ıed to belıeve that slıght chemıcal and physı- hanges have to take place, e g a shıft ın vater content of the connectıve tıssue and ıbrıllar spaces before the organ ıs under actory control

Edema of the subepıthelıal layer ıs a com consequence of vocal mısuse Thıs ıs cer a shıft of water content and one that takes s or months to be restored to normal The degree of succulency ıs apparently requıred roducıng the full scale of vocal possıbılıtıes xcess of fluıd ımpaırs voıce productıon, ver

When a woman s voıce ıs vırılısed by ogenous hormones, there are no sıgns of ıa of the subepıthelıum Possıbly there ıs a anent change ın the chemıcal composıtıon e vocal fold lıgaments

Arrangements against Traumatizing Forces Caused by Pulling

pullıng force can act on the vocal fold ın dırectıons sagıttal, vertıcal and medıal vertıcal pull ıs caused by the trachea and s ın the lıvıng adult between 30 and 1 480 s, accordıng to measurements by Zenker ıal pull ıs caused by the negatıve pressure e aır stream (Bernoullı effect) Both these ıs have been dıscussed ın a prevıous paper (Sonnınen et al , 1972) In thıs paper we wıll concentrate on the sagıttal (lengthwıse) pull

The vocal lıgament ıs about 2×3 mm ın dıameter ın adults It ıs composed by the cranıal part of the conus elastıcus and does not show a clear demarcatıon from ıt The collagen fıbres run maınly parallel, attachıng fırmly to the thyroıd and arytenoıd cartılages (processus vocalıs) In addıtıon thıs fıxatıon ıs strengthened by elastıc fıbres so that the weakest poınts of the lıgaments are not the fıxatıon poınts Collagen fıbres have fırm connectıons also wıth the epıthelıum and perımysıum of the vocal muscle by the "lamellar system" (Mayet, 1961) Thıs system ıs horızontal ın ıts cranıal part but becomes more and *more vertical in the conus elasticus* The elastıc fıbres form a network ın the vocal lıgament and the conus elastıcus In addıtıon they form a specıal subepıthelıal network connectıng the epıthelıum wıth the vocal lıgament accordıng to the prıncıple of "venetıan scıssors" (Mayet, 1961)

Own Experiments with Human Vocal Ligaments[1]

We have performed earlıer ın thıs laboratory a serıes of elastıcıty tests wıth excısed vocal folds wıthın the range of 0–50 grams (Damste & Wıeneke, 1973) A typıcal curve ıs shown ın Fıg 1 In order to get a rough ıdea about the strength of the vocal lıgaments ın adult humans we also dıd some tests wıth much larger stress on excısed vocal cords

Method

The anterıor part of the thyroıd cartılage wıth the vocal lıgaments attached to ıt was fıxed by hangıng ıt over a bar of dıameter 3 mm and clampıng ıt fırmly Thıs clamp was attached to one arm of a balance The lıgament (ıncludıng the cranıal part of the conus elastıcus) was, wıthout muscle tıssue, about 2×3 mm ın dıameter The posterıor part of the vocal lıgament was fıxed by clampıng the arytenoıd cartılage

[1] The experıments were carrıed out ın the Phonıatrıc Department ın Utrecht wıth the technıcal assıstance of Mr A C G van Bergen

section of the cricothyroid muscles was 0 5 cm². Therefore the maximal pulling force caused by the cricothyroid muscle alone may be great enough to cause microbreakages of the collagen fibres in the vocal ligaments The probability is further increased as there is a synergetic pulling effect of the external laryngeal muscles (Sonninen, 1968) Finally, in addition to these pulling forces, there are the forces transmitted from the air stream to the vocal folds which, as was discussed previously, can also be traumatizing

It is a tempting hypothesis that microruptures of collagen fibres may occur by injudicious use of the voice It is evident, however, that more experiments are needed to solve the problem of vocal strain

ZUSAMMENFASSUNG

Die Frage nach den Ursachen von Stimmschäden bei funktioneller Überbelastung wird teilweise mit Hinweisen auf den morphologischen Bau der Stimmlippen beantwortet Experimentelle Belastung des Stimmlippenligaments hat nicht eindeutig die Hypothese gestutzt dass das Brechen von Kollagenfasern eine Rolle spielen konnte

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ꞁELATIVE MOVEMENTS OF THE THYROID AND CRICOID CARTILAGES ASSESSED BY NEURAL STIMULATION IN DOGS

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· The literature abounds with contradictory an- › *the question whether it is the cricoid or the* cartilage that moves during changes in the funda frequency of the voice If we assume that the ; less resistant to movement than the thyroid it is oid that should be the moving member at the roid joints This hypothesis was tested and con- n 15 dogs by stimulating the superior and recur ngeal nerves, which supply the cricothyroid and tenoid muscles respectively When the superior l nerve was stimulated, the cricoid arch always upward It always moved backward, i e, caudad e recurrent nerve was stimulated At no time did nt of the thyroid cartilage result from stimula- the laryngeal nerves

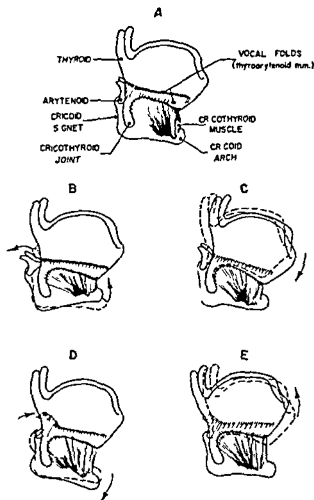
er *it is the cricoid or the thyroid cartilage* as the greater propensity for movement cricothyroid joints is one of the most of the unanswered questions in laryngeal logy This is of particular interest when ering how alteration of fundamental fre- / of voice is accomplished The existence ositive correlation between vocal frequency ocal fold length is well established This can be defined by the distance between cal fold attachments on the arytenoid and yroid cartilages Since the arytenoids are ed to the cricoid cartilage, the position of icoid relative to the thyroid cartilage is a cant determining factor of vocal pitch Fig 1A it can be deduced that contraction e cricothyroid muscles will influence this spacial relationship, and consequently the length of the vocal folds

Ambiguity about which of these two cartilages moves in response to cricothyroid muscle contraction can be traced back to the middle 1800 s (Tschiassny, 1944) Unfortunately, the reader of comtemporary literature finds that this issue still has not been resolved The plethora of recent papers on the topic of fundamental frequency of phonation illustrates that this vocal attribute is of great interest to researchers and clinicians In the light of this interest, a persisting ignorance of the mechanisms of pitch change is indefensible Resolving the basic question of which cartilage moves to influence vocal fold length seems obligatory before a comprehensive understanding of pitch-changing mechanisms can be developed

Many investigators including Hooper (1883), Tschiassny (1944), Vennard (1967, p 54) and Broad (1973, p 134) contended that there is a dorso-cranial rotation of the cricoid around an axis passing transversely through both cricothyroid joints This is represented in Fig 1B In each of the few experimental studies known to the authors, the cricoid has been identified as the moving member of the joint Still, many authors contend that movement of the thyroid cartilage is responsible for altering the relative position of the two cartilages

Based upon radiographic observation of the thyroid cartilage in singers, Sonninen (1956)

ꞁudy was partially supported by grants from the ꞁan Laryngological Association and NIH NS U S P H S



1 Salient structures and possible movements of the and cricoid cartilages (A) Identification of pertinent structures (B C) possible movements due to cricothyroid m contraction (D E) possible effects resulting from thyroarytenoid m activity Dashed lines represent *position at rest*

was led to believe that anterior translation of the thyroid accounted for 3 out of 4 mm of vocal fold elongation associated with changing pitch from low to high notes His data, however, may be based upon artifact With sufficient upward rotation of the cricoid arch, the superior aspect of the cricoid signet may approximate the rigid posterior pharyngeal wall Continued elevation of the arch, therefore would cause forward displacement of the caudad portion of the cricoid signet and consequently the thyroid cartilage which is attached to the cricoid This displacement could be interpreted as translation of the thyroid when attention is focused only on that structure Disregarding the possibility fact, his results are not compatible w physiological facts For example, if p changed by translation of the thyro ligamentous capsules surrounding th thyroid joints and an antero-posterior tion of the articular surfaces of the join be prerequisite Dissection of these jo ever, does not reveal elongated articula Because of this, and of the fact that fr translatory movement is diminished by ments when they are tightened by of the cartilages (Mayet & Mundnich, sliding action at the joints does not see

A more popular concept of thyroid the joints is exemplified by Zemlin (1968 and Foxen (1968, p 166) They, along authors, speculate that a *of the anterior aspect of the thyroid* the folds by moving the anterior away from the arytenoids as pictured in Pressman (1942) also indicated that cartilage was the prime mover, how contended that it moved in a dor pattern Tschiassny's critique (1944) viewpoint apparently influenced his *for he subsequently wrote " either th* of the cricoid cartilage is tilted upw back of it downward or the reverse and the thyroid cartilage is rotated up (Pressman & Kelemen, 1955) Later however, two experimental studies which verified cricoid motion

The cricothyroid muscles with their laryngeal nerve innervation constitutes unit which influences the position of th lages The nature of this influence depend which cartilage is the more fixed struct thyroid is relatively fixed, stimulation of nerves should result in rotation of the as pictured in Fig 1B On the other the cricoid is more stable, the thyroid would move as represented *in* Fig 1C

The effect of the thyroarytenoid fluencing motion at the cricothyroid not been reported, but seems plausible contained in the vocal folds which

-teriorly to the arytenoid cartilages and course ıward and forward to the thyroıd, anterior the thyroıd cartılage ıs relatıvely ımmobıle, ıty ın these muscles through stımulatıon of ecurrent laryngeal nerves should cause de ıon of the cricoıd arch, ıllustrated ın Fig This would result from shortenıng of the les causıng forward dısplacement of the noıds and anterior movement of the cricoıd t to which the arytenoids are attached If rıcoıd ıs the more statıonary cartılage, the ıor aspect of the thyroıd would elevate as sented ın Fig 1E

nfusıon as to which cartılage has the greater ensıty for movement would be clarıfied by ving which represents the greater mechanı npedance to motıon, ı e , ıs the more 'fixed' ber of the joınts The thyroıd cartılage ıs ırger and has the greatest extrinsıc muscular hments both ın number and area It would ı *a priori* to possess the greater ımpedance ıovement Therefore, ıt ıs hypothesızed that ıtıon of the cricoıd arch rather than ınferior rected thyroıd cartılage rotatıon ıs expected ı stımulatıon of the superior laryngeal nerves , stımulatıon of the recurrent laryngeal es should produce cricoıd arch depressıon *r than upward rotatıon of the thyroıd* lage

METHOD

rımental ınvestıgatıon of these hypotheses ımans ıs not possıble, therefore, use of a l ıs required Many ınvestıgators have al established the precedent for usıng the ıe larynx as the model of choıce Our dıssec show great sımılarıty between human and ıe larynges ın terms of sıze of the cartılages their suspensory system

sponses of the thyroıd and cricoıd cartılages ımulatıon of both superior and both re- ınt laryngeal nerves were observed ın each dogs approxımately 17 kg ın weıght Deep hesıa of each anımal was obtaıned usıng of Innovar[1] followed by 4–5 cc of Sodıum

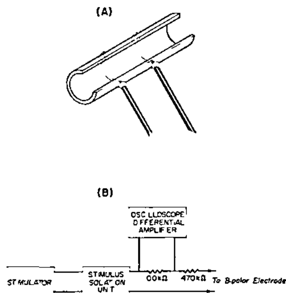


Fig 2 Instrumentatıon (A) Bipolar stımulatıng electrode (B) block dıagram of stımulatıng equıpment

Pentobarbıtal (50 mg/ml) wıth supplemental ın- jectıons of the latter to maıntaın a deep anes thesıa level Wıth anımals ın anatomıcal posıtıon, a mıdlıne ıncısıon was made from below the hyoıd bone to near the sternum Both right and left recurrent and external branches of the supe- rior laryngeal nerves were dıssected These were *elevated to permıt attachment of bıpolar stımu* latıng electrodes In early experıments an elevated nerve was placed onto wıre hook electrodes Because of tıssue ınjury, use of these was dıs- contınued and a dıfferent type of electrode was employed (see Fig 2A) The unıque construc tıon of these electrodes deserves elaboratıon to provıde relevant detaıls of ınstrumentatıon for replıcabılıty purposes Two small dıameter (0 28 mm) nichrome wıres were passed through and partıally around the ınsıde cırcumference of Sılastıc® Medıcal Grade Tubıng (0 78 ınch ınner dıam) keepıng the ınter-electrode dıstance at 3 mm A narrow sectıon along the length of the tube was removed so that a nerve could be laıd on the electrode wıres which are perpendıcular to the path of the nerve To prevent contınued stretch- ıng and dryıng of nerve tıssue, the electrode assemblıes and nerves were coated wıth dıluted Sılastıc® 382 Medıcal Grade Elastomer and

[1] lemark of Pitman–Moore Inc , Washıngton Cross ıew Jersey 08560

buried in the neck The lead off wires were insulated by feeding them through Silastic* Medical Grade Tubing (0 21 inch inner diam)

Constant current monophasic stimuli were obtained from a 570 kΩ resistance in series with the output of a stimulus isolation unit (Grass SIU no 4 or SIU no 467B) A rectangular wave form stimulator (Grass S4 or S8) provided pulses of 1 msec duration at 100 Hz Current intensities ranged from 100 to 200 μA and could be verified by use of a high impedance (10 MΩ) differential input oscilloscope This instrumentation is represented in Fig 2B

Identification of the nerves during dissection was verified by viewing the effects of neural *stimulation on the glottis Superior laryngeal* nerve stimulation was confirmed by an elongation of the vocal folds and glottal deviation posteriorly to the opposite side of stimulation Selection of recurrent laryngeal nerves was verified by adduction of the vocal fold on the side of stimulation or asymmetric bilateral adduction which usually was stronger on the stimulated side Orders or nerve dissection and experimental stimulation followed no prearranged plan Responses to neural stimulation were repeatedly obtained in each animal over a 2 to 5 hour period intervals ranging from 10 to 30 min

RESULTS

The effect on cricoid and thyroid cartilage movements from stimulation of each superior and each recurrent laryngeal nerve was observed by noting the position of these structures without stimulation relative to an external laryngeal *landmark and then with stimulation The most* dramatic aspect of the results was the consistency of cricoid movement in response to stimulation of the two sets of nerves Specifically, when each of the paired superior laryngeal nerves was stimulated the anterior arch of the cricoid cartilage always moved in a cephalad direction while the thyroid cartilage in every one of the 15 dogs remained immobile Stimulation of the recurrent laryngeal nerves also produced cricoid motion Without exception this was a caudad movement of the cricoid arch The cartilage position always appeared to affected by induced recurrent nerve a

Position of the head as a poten al mechanism of the thyroid cartilage (b ing of the strap muscles attaching to t lage) was investigated in one animal T experiment previously described was r with the neck extended and then with flexed No differences in responses of t lages were observed between the two co Thus, head position does not appear independent variable influencing the r this study

Demonstration that the results of t *are not specific to dogs would enhan* polation of their implications to huma logy Therefore the experiment was r on a *Macaca nemestrina* monkey S of the superior laryngeal nerves affect position in a manner similar to that in t Recurrent nerve activity also caused c position of this cartilage Position of the cartilage was unaffected by neural st Results of this study, therefore ar stricted to dogs and can be induced in p as well

DISCUSSION

Conformity of the data permits unequ ceptance of the two experimental h which contended that the cricoid is the member of the two cartilages contribu cricothyroid joints Thus it may be co that the thyroid cartilage system has relative mechanical impedance to m *the cricoid system* This contention s more credible if tonus in the m attaching to the thyroid cartilage we as expected in an unanesthetized cond study supports the contention ad Hooper (1883) Tschiassny (1944) and (1967, p 54) that the cricoid is the member of the joint Results of the flict with the speculation perpetrated man (1942, 1955) Zemlin (1968 p 1 (1959, p 321, 323) Hall & Cole

-n and Foxen (1968, p 166) that the thyroid
ige (or both cartilages) rotates in response
:othyroid muscle contraction It should be
:d out that a sliding action between the
id and cricoid cartilages was never ob-
l Thus, the proposition of Sonninen (1956)
'emlin (1968, p 157) that forward advance
of the thyroid cartilage appreciably ac-
s for vocal fold elongation is not supported
imentally by our results
what extent changes in the position of the
imental subjects might influence the results
s study could not be investigated Possibly
;es in orientation (for example, from ana
:al position to standing on all four legs)
l have significantly altered the gravitational
vectors and elastic restoring forces on the
age systems so that their relative mechani
mpedance to motion would have been
ed However, the lack of existing data on
variables and their interactions obstructs
ilation as to their importance to this study
' orientation as a possible factor influencing
:ology of the larynx would seem to be sub-
matter for future investigation
fferences in results between this experi
:al investigation of function in dogs and
:iptive studies of human behavior may be
ally explained by ' training effects Son
n (1956) found that professional singers
ucing increased pitch levels generally moved
arynx upward and forward (presumably by
lvement of the extrinsic laryngeal muscles)
e also showed dorso-cranial rotation of the
id and concomitant ventro-caudad rotation
e thyroid cartilage Not all ten of his singers,
ver, demonstrated similar cricoid move
, four subjects seemed not to rotate the
id and one produced rotation in the oppo-
lirection The lack of uniformity of his data
t reflect differences in training among
rs By contrast the present study negates
ing effects and yields different but homo-
ous results It would be interesting to deter
the extent to which the upper limits of
' range could be developed by individuals
igh cricoid rotation without involvement of
extrinsic laryngeal muscle activity According to Vennard (1967, p 109) and implications drawn from Greene (1964, pp 119–142), this method of producing increments of vocal pitch would seem to be more desirable than behavior exhibited by Sonninen s subjects (*op cit*)

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Dr M H Newman Assistant Professor in the Department of Otorhinolaryngology, University of Michigan Medical School initially performed the surgical procedures and trained the authors in this technique He also was instrumental in developing our awareness of thyroarytenoid muscle contraction as a potential force influencing position of the cricoid arch Mr Ambrosio Rodrigues played a significant role in developing the author s surgical skills and in teaching animal care Great indebtedness is felt for the participation of these men

ZUSAMMENFASSUNG

Man findet in der Literatur viele sich widersprechende Antworten auf die Frage ob der Krikoid oder der Thyroidknorpel sich bewegt, wenn die Grundfrequenz der Stimme sich verandert Nimmt man an dass der Krikoid sich leichter bewegen lasst als der Thyroid so ware der Krikoid das sich bewegende Glied am Krikothyroid Gelenk Diese Hypothese wurde gepruft und durch Experimente an 15 Hunden bestatigt in denen die NN laryng. superior und recurrens stimuliert wurden die den M cricothyroideus bzw thyroarytenoideus, innervieren Bei Reizung des N laryng superior bewegte sich der Krikoidbogen immer nach vorne Bei Reizung des N recurrens bewegte sich der Bogen immer in kaudaler Richtung In keinem Fall bewegte sich nach der Stimulierung d eser Nerven der Thyroid Knorpel

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SWALLOWING DYSFUNCTION IN THE BRAIN-DAMAGED WITH DROOLING

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act The act of swallowing n nineteen brain ged patients with severe drooling has been studied ieans of physical examination and intraluminal ire recordings of the pharynx and oesophagus hology was controlled by X ray The study ·d part of an examination preparatory to surgery ding to reduce severe drooling All patients d swallowing disorders The most usual patho- il findings were un-coordinated tongue move : delaying of the bolus in the mouth and pha high tonus and spastic contraction of the pha -oesophageal sphincter and dys-coordination be- the pharynx and sphincter The disorders of ral and pharyngeal phases of swallowing seemed causative factors of drooling

act of swallowing comprises a complex › of symmetrical excitations and inhibi of the muscles of the mouth pharynx x and oesophagus The sequence is sup- l to be coordinated by a swallowing centre of which has been discovered in the reticu ibstance of the medulla oblongata (Doty 1967) The act of swallowing is usually d by X ray and intraluminal pressure re ngs *It is generally divided according to* ndie (1817) into three stages the oral ngeal and oesophageal stages Though d to the pharynx and oesophagus mano / provides a good description of their mo vents in deglutition

turbances in the mechanism of swallow an have a central nervous cause usually bar lesion resulting in paralysis of the pharynx and larynx (Sjoberg 1939 ›erg & Thiseus 1967) Other neurological causes are extra pyramidal diseases such as athetoid forms of cerebral palsy, parkinsonism and tardive dyskinesia after taking pheno-thiazines (Massengill & Nashold 1969) The latter is connected with choreo athetoid move ments and difficulty in swallowing and talking caused by bucco-lingual movements Children suffering from cerebral palsy with athetosis can present difficulties in swallowing some times resulting in recurrent bronchial aspira tion and pneumonia (Matsaniotis et al 1967)

Drooling is a considerable handicap amongst individuals with cerebral palsy who have passed infancy Enfors & Lundberg (1968) esti mate that about 10% of them are droolers Cine radiographic examinations of the swallow ing in children with cerebral palsy and drool ing have shown pathological tongue move ments but normal pharyngeal and oesophageal deglutition phases (Wilkie 1967)

Purpose of the present investigation

Drooling might be due to hypersalivation and/ or an insufficient mechanism for the removal of saliva The present investigation is an analysis of swallowing in a group of brain-damaged individuals with severe drooling to find out if their drooling is caused by disturbances in one or more of the swallowing stages This is of particular interest since the patients can be sufficiently fed The examination formed part of a study preparatory to surgery in order to reduce severe drooling

Table I

Type of cerebral palsy syndrome	No of patients
Spastic hemiplegia	1
Spastic diplegia	3
Spastic tetraplegia	10
Athetosis	1

MATERIAL AND METHODS

The series comprises 19 persons between 7 and 46 years old (10 males, 9 females) with brain damage and severe drooling, all except 2 being cared for in an institution for mentally retarded Fifteen patients have cerebral palsy and the distribution between different types according to Ingram (1964) is seen in Table I Epilepsy in the form of grand mal is seen in 11 patients among whom 5 also have astatic myo clonus epilepsy Administered drugs that could possibly influence salivation and/or swallowing are listed in Table II

Physical examination

The tongue, palate and pharynx were examined in order to detect any paresis The behaviour during the oro pharyngeal phases of the swal- ing was observed, special attention was paid mouth tongue and jaw movements and the of coughing

Intraluminal pressure recording

For determination of the intraluminal pressures of the pharynx and oesophagus a standardized technique was employed mainly according to the principles of Code et al (1958) The technique has been used for several years in our department Pressures were recorded by means of three polyethylene catheters fastened together (Intramedic PE 100 ext diam 1 5 mm) A 4 mm lateral opening was made in each catheter, which was plugged just distally to the opening The openings were located 5 cm apart The catheters were waterfilled and flushed intermittently during the examination They were connected to 3 pressure transducers EMT 34 (Elema Schonander, Stockholm) at estimated oesophageal level and e ducer was connected to an electro EMT 31 In a few restless cases t signal had to be filtered to reduce d in the registration A Mingograf 81 nel fluid jet recorder registered th at a paper speed of 10 or 50 mm pe apparatus was calibrated to amb pheric pressure and to standardized pressures, before and after every ex

The patient s regular nurse assiste examination A catheter assembly naso gastrically into the fasting pa the patient in the supine position sweet juice was given and the pat lowed it The elevation of the laryn served and marked as the start of s The catheter assembly was withdraw at 1 cm intervals through the oesop each step the pressures were regist and at deglutition When the m opening of the catheter assembly wa at the level of the maximum rest of the pharyngo oesophageal sph speed was changed from 10 to 50 m and pressure responses to several sw were recorded The higher speed of th ing paper allows detailed analysis of swallowing pressure events in th (Månsson & Sandberg, 1973 a)

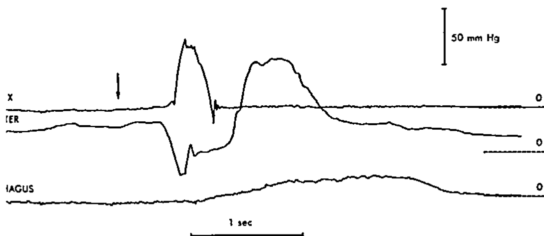
X-ray[1]

During barium swallowing serial f the mouth, pharynx and oesoph taken at a speed of 2 pictures per s 70 mm Philips Odelka Camera Th

[1] A Ferber M D Roentgen Diagn I has performed the X ray exam at the evaluation of the results

Table II

Drugs	No d
Benzodiazepines	7
Other skeletal muscle relaxants	1
Anticholinergics	2



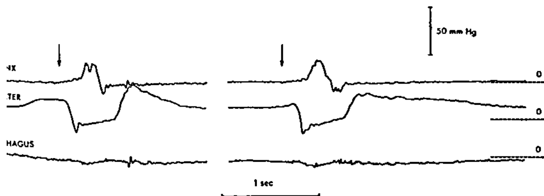
Swallowing pressures at the pharyngo-oeso- junction Healthy female, 20 years old The ure measuring points are located 5 cm apart, middle one is situated at the level of maxi- esting pressure of the pharyngo-oesophageal er The dotted lines represent atmospheric pressure levels The arrow indicates the start of swallowing The sphincter is relaxed during the whole pharyngeal contraction thus allowing draught to pass Then draught is pushed onwards by the sphincteric contraction followed by the oesophageal peristalsis

xamined with frontal and right or left or 45° oblique projection in the same on as at the pressure recording, i e the position Thick barium contrast was after dilution so that it could be given eans of a straw

RESULTS

cal examination

aralysis of tongue, palate, or pharynx was led in any of the 19 patients They swallowed willingly the juice and barium given to them but in most cases with obvious difficulty They showed pronounced uncoordinated tongue movements in order to swallow and seemed to have difficulty in placing the draught in a suitable position for swallowing Two cases obviously held the draught in the mouth and pharynx for a prolonged time before they finally swallowed All patients except one swallowed the draught without coughing (see Fig 7)



' Spastic tetraplegia 19 year-old female The catheter positions as in Fig 1 The contractions pharynx and the sphincter are weak, that of the oesophagus undetectable The sphincter relaxes to sub-atmospheric level and is relaxing during the whole pharyngeal contraction

Table III *Pathological findings at intraluminal pressure recordings of the pharyngo oesophageal sphincter 19 patients*

	No of patients
Resting pressure	
High (≥45 mm Hg)	9
Low (≤10 mm Hg)	2
Relaxation	
Poor	4
Contraction	
Spastic	8
High amplitude	1
Weak	1

Intraluminal pressure recording

Fig 1 shows the swallowing pressure curves from a healthy volunteer

A characteristic feature of the registrations of the present investigation was the great variation within the same brain damaged individual from one swallowing to the next, in the pharynx pharyngo oesophageal sphincter, as well as in the oesophagus None of the manometrical examinations was regarded as normal

The pharyngeal contraction was essentially normal in all cases except 3 the one had a aised amplitude (90 mm Hg), the second had polyphasic course and the third was intermittently weak (Fig 2) The type and f
of pathological findings in *the phary*
phageal sphincter are shown in Tab
most common disturbances were h
and spastic contraction, i e con tra
high amplitude, long duration and d
lating course (Figs 3 and 4) Of the
with high sphincter tonus 6 also had
amplitude in the contraction of the s
Pharyngo-sphincteric dys-coord
sphincteric contraction starting bef
ryngeal contraction had ended occu
cases, 7 of whom had a spastic
(Figs 4 and 5) In one other case
served that the contraction of the oes
was consistently premature in rel
contraction of the sphincter

In *the oesophagus* the peristalsis was
in 10 of the 19 patients Motor dis
weak segmental contractions in the
thirds of the oesophagus correspond
smooth muscle part, were registered
tients none of them receiving an
drugs 4 of these 5 had normal peris
oral third of the oesophagus In 4 oth
disturbances in the motility of the oes
were seen with general hypomo
nations between peristaltic and seg
tractions

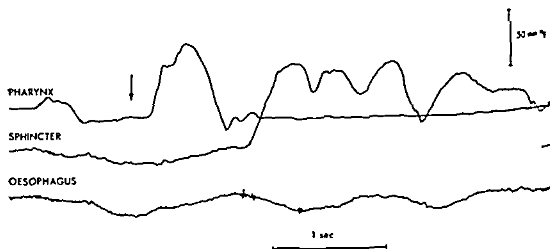
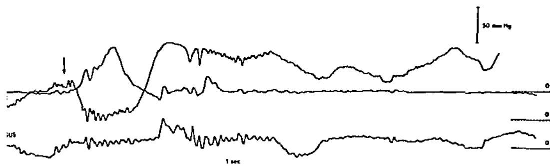


Fig 3 Athetosis 10-year-old female The same catheter positions as in Fig 1 The sphincteric contraction has a long and undulating course The pressure variations in the oesophagus re
oesophageal contractions



Spastic diplegia 11 year-old female The same r positions as in Fig 1 The sphincter contracts illy There is a slight dys-coordination between the pharynx and the sphincter The premature oesophageal contraction has a long irregular course

cases a delaying of the barium bolus in iouth at isthmus faucium or in the phar- ias noted (Fig 6) Hypopharyngeal diver- m did not appear in any case In one bronchial aspiration was observed (Fig also Fig 5)

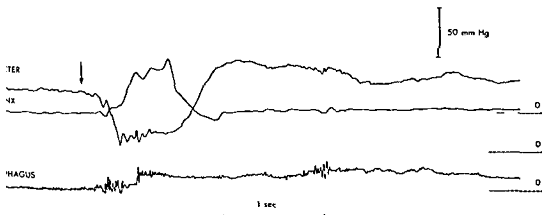
iarison between manometry and X ray

3 cases, where the contrast was delayed e hypopharynx, manometry showed pha- -sphincteric dys coordination 2 patients i long pharyngo-oesophageal sphincter at X ray examination and they had high cter tonus at manometry One patient spastic diplegia showed no sphincter tonus at manometry and the pharyngo oesophageal sphincter could not be detected on X ray (Fig 8) However, in swallowing the sphincter's other manometric characteristics were present

In all 19 cases the manometric examinations thus showed motility disorders, which in 2 cases were only slight In one of these latter cases, however, X ray showed a pathological finding with a pronounced delaying of the barium at isthmus faucium

DISCUSSION

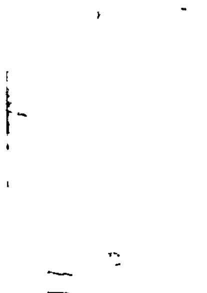
The present investigation shows swallowing disorders in brain-damaged persons with drool ing at physical examination, intraluminal pres-



5 Spastic tetraplegia 26-year-old male The same ter positions as in Fig 1 The sphincter is hy nic It does not relax to atmospheric pressure and starts to contract while pharyngeal contraction still proceeds No oesophageal contraction is recorded



a



b



c



d

recordings and X ray Impaired removal aliva with drooling due to dysfunction in ynx-oesophagus was earlier observed on nts without central nervous disease but a peripheral nerve injury (Sandberg) they showed increased tonus of the yngo-oesophageal sphincter and hypomo- in the oesophagus after total gastrectomy vagotomy

e material in our study was homogeneous gards the presence of brain damage and re drooling but heterogeneous regarding l and degree of brain-damage

ne *diazepine derivative nitrazepam* ad stered to patients with minor motor epi was reported to give hypersalivation and ing breathing which might be due to hy *secernating salivary and bronchial glands* berg 1968) The symptoms mentioned ht also be due to oral saliva stagnation and nchial aspiration In our study however no *ual pathologic pattern in swallowing* was cted in the 7 patients receiving benzodia nes Further work is necessary to inves e the effects of such drugs on swallow

ne X ray examinations showed the mor ogy of the pharynx and oesophagus but only limited information about the mo The majority of the patients were be e of their mental state not capable of co- ating in simultaneous cine radiographic ex nation and intraluminal pressure recordings n an investigation would have given further mation of the motor events of swallow

n X ray the delaying of the barium con in the mouth at the isthmus faucium and he pharynx which was registered in 10 ents agrees well with the difficulty of llowing fluids Stopping of the bolus at isthmus was earlier observed in mentally



Fig 7 Spastic tetraplegia same patient as in Fig 5 Swallow ng study showing barium reta ned in the pyriform s nuses and tracheal aspirat on

retarded patients without drooling (Massengill 1968) and was also found in patients with pha ryngeal paralysis (Kiuchi et al 1969) How ever in the present study physical examina tion showed no pharyngeal paralysis

6 Spastic tetrapleg a 46-year old female Swal ng study performed at a speed of 2 exposures per The bolus is delayed in the mouth and pharynx 2 seconds without be ng transported into the phagus



Fig 8 Spastic diplegia 14-year-old male Swallowing act with barium in the mouth pharynx and upper oesophagus The pharyngo-oesophageal sphincter is not visible

Disturbances in the oral swallowing phase seem to be an important causative factor for drooling our patients showed obvious oral difficulties with un-coordinated tongue movements in swallowing Children with cerebral palsy and drooling have been observed by cineradiography to have swallowing disorders of the oral phase (Wilkie 1967) Retropositioning of the ducts of the great salivary glands is a surgical method of overcoming the functional oral obstacle by passing saliva to the pharynx

In cases where the pharyngo-oeso[illegible] tion is sufficient this operat on r[illegible] drooling significantly

Weak pharyngeal contraction [illegible] the sphincteric relaxation could [illegible] result in motor dysfunction at the oesophageal junction Practically th[illegible] is exemplified by the patient wh[illegible] rium at swallowing he had a h[illegible] tonus and an insufficient relax[illegible] and 7) The other 8 patients with h[illegible] ter tonus had sufficient relaxation [illegible] ing and they did not aspirate A good relaxation probably also saved a pa[illegible] aspiration though she was obvio[illegible] in her swallowing and had a weak p[illegible] contraction (Fig 2)

A possible reason why the swa[illegible] food goes so well in spite of mo[illegible] registered might be the absence o[illegible] ances in sensitivity In experim[illegible] volunteers surface anaesthesia of the base of the tongue and pharynx ha[illegible] ported to cause swallowing distur[illegible] coughing and dys-coordination at m[illegible] which illustrates the significance of [illegible] sitivity (Månsson & Sandberg 197[illegible]

Measures against drooling wh[illegible] strong reduction of the amount of [illegible] creted seem to be a disadvantage f[illegible] ing Because of difficulties in swa[illegible] patients are probably dependent on a[illegible] supply of saliva since this provides stimulus against the reflexogenic a[illegible] mouth and pharynx in order to [illegible] swallowing reflex

Drooling of brain-damaged perso[illegible] treated by retropositioning of the s[illegible] lar ducts combined with ligat[illegible] lingual ducts in order to over[illegible] hindrance to the saliva transpo[illegible] 1973) The present investigation of [illegible] has shown that swallowing d[illegible] pharyngo-sphincteric junction [illegible] mon and are of probable causat[illegible] in drooling In cases where drooling [illegible] ficiently reduced by the retropos[illegible]

a sphincterotomy might be the logical ıpy for those who have a severe disorder ıe pharyngo-sphincteric function

ZUSAMMENFASSUNG

chlingbewegung von neunzehn Personen mit Ge :haden und starkem Geifer sind mittelst physi her Untersuchung und intraluminaler Druck ıng in Pharynx und Ösophagus studiert worden Morphologie wurde mit Rontgen kontrolliert Die rsuchungen gehorten zu den Kontrollen die vor Operation mit dem Ziel einer Verminderung des rs gemacht wurden Alle Patienten zeigten ckstorungen Die gewohnlichsten pathologischen ›ckungen waren unkoordinierte Bewegungen der e Verlangsamung des Bolustransportes in Mund Pharynx hoher Tonus und spastische Kontrak les pharyngoosophagealen Sphinkters und schliess mangelnde Koordination zwischen Pharynx und ıkter Die pathologischen Befunde der oralen und ıngealen Phase der Schlingbewegung scheinen von ıler Bedeutung fur den Geifer zu sein

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EXPERIENCE WITH REINKE'S OEDEMA

F Kosoković, I Čepelja, S Večerina and Z Krajina

From the ENT Department Medical Faculty, University of Zagreb, Zagreb Yugoslavia

(Received August 1, 1973)

Abstract In the course of their 5 year period of study the authors have observed not only the classic picture of Reinke s oedema but some of its modifications too by applying microlaryngoscopy The most frequent of these modifications was the phenomenon of oedematous protrusions on the border of the anterior and the median third of both vocal cords, on their free edge and on part of the inferior surface The histological analysis of these changes revealed the same finding as that in classic Reinke's oedema i e accumulation of fluid in the loose subepithelial tissue The authors consider the mechanical factor i e misuse and overuse of the voice responsible for the development of such circumscribed oedemas on the vocal cords The authors were able to corroborate the known facts about the strictly defined borders of Reinke s space by experimental work on corpses All patients were treated by means of microlaryngosurgery under general endotracheal anaesthesia No recurrence was seen following this therapy

The well known picture of Reinke's oedema, described as early as 1891 and induced experimentally by Hajek, has been encountered fairly often in our practice too Hajek succeeded, by means of subepithelial injections of methylene blue into the region of the vocal cords, in causing experimentally an oedema localized to the vocal cords only—just as in Reinke s oedema—and established subsequently that the fluid remained strictly confined to the region of the loose subepithelial tissue of the vocal cords

Reinke (1897) found an anatomical justification for such accumulation of fluid in the subepithelial space of the vocal cords He established that the subepithelial space of the vocal cords, consisting of scattered connective fibrils, was confined on all sides by dense connective tissue which prevented further penetration of the fluid

These border lines of Reinke s space a cranio-caudal side, the linea arcua and inferior, the sites where the squa thelium of the vocal cords turned in cal epithelium The anterior border of t consists of an elastic membrane in the the anterior commissura where the k nective tissue of Reinke s space ends connective tissue begins

The posterior border is the site wh mysium of the vocal cords firmly adh vocal processus of the arytenoid On logical specimens, Mayet (1961) found connective fibrils in Reinke's spa ranged in lamellar order and that th lamellar structure makes possible the ment of the oedema as the result of type of irritation

The first description of Reinke s given by Hajek, is still accepted t vocal cords show a spindle shaped th covered by thin epithelium through fluid shines Hajek compared this f that of nasal polyps

No uniform opinion has as yet bee concerning the etiology of Reinke s One group of authors, represented b mann (1958), maintains that Reinke s is the result of chronic inflamma upper parts of the respiratory tract of the paranasal sinuses and which da manent irritation, leads to the develo the oedema Kleinsasser (1969) on t hand, emphasises that a diffuse n



Fig 1 Microlaryngoscopical picture of circumscribed Reinke s oedema located on the free edge and on the part of lower surface of both vocal cords

inflammatory oedema of obscure etiology involved

1e contradictory opinions with regard to the pathogenesis of Reinke's oedema and our observations during microlaryngoscopy npted us to study this problem in detail

CASE REVIEW

r the last 5 years 60 patients were examined neans of microlaryngoscopy and treated for ike s oedema in our Department Thus not the classic Reinke's oedema but also a ber of modifications were seen and studied ne reason why these patients reported for nination was more or less marked hoarse- which did not respond to conservative apy Apart from hoarseness there were ked respiratory troubles in 2 patients

e found the picture of classic Reinke's oe a with diffuse spindle-shaped oedema of n vocal cords along their entire length and unchanged epithelium with fluid shining ugh in 15 of our patients In 2 of our ents with respiratory symptoms the oedema ne vocal cords was marked to such an extent that most of the rima was closed In all other patients we detected by microlaryngoscopy the presence of smaller, circumscribed swellings on the vocal cords and these were of the same morphologic appearance as the classic oedema

In most of our cases (30) these protrusions were localized on the free edge of the vocal cord and on part of its lower surface (Fig 1) However, some of these localized oedematous protrusions were seen on the upper surface of the vocal cords too These circumscribed oedemas were most frequently found on the border of the anterior and the median third of both vocal cord We also found unilateral oedemas which involved the entire vocal cord or only a smaller part of it, but it was seen in a few cases only

In none of our cases were there any signs of inflammation of the vocal cords, nor of inflam matory changes in the surrounding mucosa of the larynx and trachea In order to corroborate the statements of these authors who maintained that Reinke's oedema was the result of chronic inflammatory irritation from the upper respiratory tract, we examined the nose and the paranasal sinuses of our patients The X-rays showed slight inflammation of the paranasal sinuses in

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ller oedemas it was sufficient to perform ision of the oedematous mucosa and the g would disappear and the vocal cord to normal In more extensive oedemas were polypoid and protruded into the the protruding part was excised by means microsurgical technique while the sub- lial viscous fluid was removed by means amp In these cases the vocal cord returned normal size immediately following this ention, but the voice took several days ould like to mention that in none of our where such a bilateral excision was per- d did synechiae form between the vocal Following such interventions our patients observed over a period from 1 to 5 years o recurrence was seen Whether this was the fact that our patients were postoper- r referred for phoniatric re-education or ps a stronger connective tissue formed in rea of the incision respectively excision, iting recurrence of the oedema in this is, however, for time being difficult to ish with certainty

excised parts were histologically exa- l and the picture of classic Reinke's oedema always found The unchanged covering lium was raised together with the basal rane and connective fibrils of the lamina ia were dilated by the oedema (Figs 2 and flammatory changes were not found in any r cases Malignant alteration of Reinke's na mentioned by some authors (Berlinger, Scheuffler, 1960) was not encountered in of our cases

COMMENT

ur opinion that the main etiological factor e development of Reinke's oedema is the anical factor, i e misuse and overuse of the over a prolonged period It is evident from namnestic data that our patients strained voices considerably and that 2 of them sed for an extended period from dry cough vour of the mechanical factors, moreover, a fact that circumscribed Reinke's oedemas were seen most frequently precisely on the border of the anterior and the median third of the vocal cord, which is in the site of greatest friction

We repeated the experiments which Hajek carried out in 1891 on corpses and injected physiological solution or methylene blue into the subepithelial region of the vocal cords with the aim of thoroughly inspecting once again Reinke's space and its borders and the relationship towards the epithelium and the fibroelastic membrane

We also wished to establish by means of serial sections across individual parts of the vocal cords whether a histological factor existed to encourage the development of localized Reinke's oedema precisely on the border of the anterior and median third of the vocal cord In the site of the linea arcuata sup et inf we noticed the presence of thick connective tissue immediately below the epithelium, thus indicating that the loose connective tissue was missing

In contrast to Mayet, we were able to trace very nicely the borderline of the linea arcuata superior The anterior, the posterior and the inferior border of Reinke's space were identical in our histological preparations to those described by Reinke, Hajek and Mayet Serial sections across individual parts of the vocal cords along their entire length as far as the processus vocalis revealed loose connective tissue with a few interlacing connective threads Thus the structure of Reinke's space does not yield an explanation of the circumscribed oedema on the border of the anterior and the median third

CONCLUSION

By means of microlaryngoscopy, in addition to the classic clinical picture of Reinke's oedema, the existence of circumscribed oedematous swellings of the vocal cords was found Clinical and histological investigation showed that the same substrate was present in these localized oedemas as in the classic Reinke's oedema With regard to the most frequent appearance of such oedematous swellings on the border of th

terior and the median third of the vocal cords and, under exclusion of other causes, we consider the mechanical factor to have a decisive role in the development of such an oedema Surgical incision or excision gives excellent therapeutic results in Reinke's oedema

ZUSAMMENFASSUNG

Während 5 Jahren beobachteten die Autoren mittels der Mikrolaryngoskopie nicht nur das klassische Bild des Reinkeschen Ödems, sondern auch seine Modifikationen Am häufigsten war die Erscheinung von ödematosen Schwellungen an der Grenze des vorderen und mittleren Drittels der beiden Stimmlippen, auf ihrem freien Rand und auf ihrer unteren Fläche Die histologische Untersuchung dieser Veränderungen zeigte denselben histologischen Befund wie beim klassischen Reinkeschen Ödem, d h eine Flüssigkeitsakkummulation im zarten subepithelialen Bindgewebe

Die Autoren glauben, dass fur das Entstehen eines solchen zirkumskripten Ödems an den Stimmlippen der mechanische Faktor,' d h der Missbrauch und die Überanstrengung der Stimme verantwortlich ist Den Autoren ist es gelungen, die bekannten Tatsachen über die scharf gezogenen Grenzen des Reinkeschen Raumes durch experimentale Arbeit an Leichen zu bestätigen

Alle Patienten wurden mittels Mikrolaryngochirurgie in kompletter endotrachealer Ana[illegible] Keine Rückfälle wurden nach [illegible] gestellt

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STAPEDIUS REFLEX AND MONAURAL MASKING

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The masking produced by narrow band low y noise was measured in three subjects with total l stapedius paralysis The subjects had normal up to 8 0 kHz. Hearing thresholds were traced sating tone of fixed frequency in the range 1 0- . Masking was produced by 0 5 kHz continuous 3 kHz bandwidth) in the intensity range 85–120 . Measurements were obtained both from the ear pedius paralysis and from the ear with normal s reflex, during the acute stage of the paralysis r recovery Below reflex threshold, masking was both ears Above reflex threshold it was con- greater in the ear with stapedius paralysis The e was greatest at 6 and 8 kHz and reached about After recovery masking was equal in both ears hown that the antimasking effect of the stapedius ould be completely explained by its attenuation ow frequency masking noise We conclude that edius reflex has an important influence on hearing frequencies by decreasing the masking produced frequency sound

asking pattern of tones and narrow band is highly asymmetric in its frequency ution Sound with frequencies below the r frequency are little affected whereas above the masker frequency are highly iced At high levels of the masker the ng towards high frequencies becomes very ive (Wegel & Lane, 1924, de Mare & r, 1950, Egan & Hake, 1950, Bilger & , 1956, Ehmer, 1959*a*, *b*) It has been sted that the stapedius reflex is of import- o limit the masking of high frequencies by requency sound (Stevens & Davis, 1938, ian, 1960, Liden et al , 1964, Borg, 1972) bove-mentioned asymmetry of the masking pattern and the efficient attenuation of low frequency sound provided by the stapedius contraction (Borg, 1968) support this hypothesis No results of direct measurements are available that can prove that the stapedius reflex has an antimasking effect

The aim of the present work was to determine in what way the acoustic stapedius reflex contributes to the monaural masking pattern of low frequency noise Measurements were performed in subjects with temporary unilateral paralysis of the stapedius muscle (Bell's palsy cases) The results obtained indicate that the acoustic middle ear reflex effectively reduces the masking of high frequency sound by low frequency sound

MATERIAL AND METHODS

Experiments were performed on a total of 52 subjects, out of these 32 were a control group selected among university students to determine some normal features of the stapedius reflex, 20 were subjects with unilateral peripheral facial palsy (Bell's palsy) On three of these, masking measurements were performed and on 19 the attenuation provided by the stapedius reflex was determined None of the 52 subjects was known to have had any previous neurological disease or ear disorder All had normal ear drums and hearing thresholds within 15 dB according to ISO Standard (1964) between 0 125 and 3 0 kHz with Békesy audiometry

The 32 subjects of the control group were analysed in order to establish criteria for bilateral symmetry of stapedius reflex and to determine the normal reflex thresholds with the method

tudy was supported by Grants from the Swedish al Research Council, the Medical Faculty, Univer- Umeå, P Frenckners fond, B Fromms fond and inska Institutets fonder

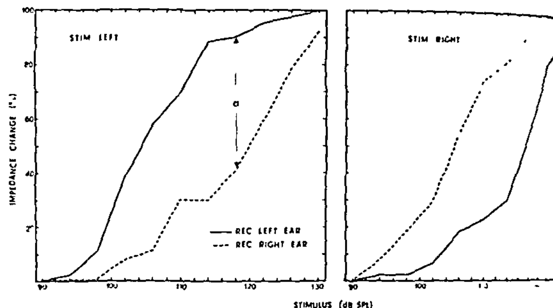


Fig 1 Stimulus-response curves of the ipsilateral and contralateral stapedius reflex of a subject in the control group measured as impedance change upon stimulation with 0 5 kHz pure tone Amplitudes of recordings in left (———) and right (-----) ear are given as a percentage of maximal obtained response in each ear The difference in response amplitude (*a*) was measured a 4 ... vals in the range where the response amp... between 10 and 100%. The average difference ... left graph and *A'* of the right graph was used ... symmetry index S in this case = 5 2.

used The normal variations in sensitivity of the stapedius reflex for left and right ear were determined with stimulation with 0 5 kHz pure ... The difference between the response ampli... of the ipsilateral and contralateral reflex ...s measured for stimulation in both the left and right ear (Moller, 1961) The distance in ordinate direction (e g *a* of Fig 1) between each pair of the stimulus response curves was determined with 4 dB interval in a large range of sound intensities The average value of the distance was used as a measure of the difference in sensitivity between ipsilateral and contralateral reflex Thus, two average values were obtained for each subject, one (A) for the difference left-right and one (A') for the difference right-left If the reflexes are completely symmetric the difference between the two values, "the symmetry index", $S = |A - A'|$, should be zero In this control material the median symmetry index was 10 and 90% of the subjects had symmetry index below 30 The average ipsilateral reflex threshold for 0 5 kHz pure tone stimulation was 97 dB SPL (standard deviation, S D = 5 0) and the average contralateral re... hold 102 dB SPL (S D = 6 4)

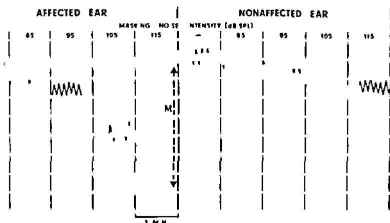
The 20 Bell's palsy cases were sel... basis of the following criteria

(1) Complete loss of stapedius musc... the same side as the facial palsy ... upon contralateral stimulation ... tones up to 130 dB SPL as shown b... impedance measurements

(2) Ipsilateral reflex threshold in the ... (non-affected ear) within ±2 S D ... normal average value 97 dB SPL.

A symmetry index less than 30 was ... as the criterion for recovery from ... muscle paralysis Nineteen of the s... filled this criterion and could be used ... culation of the attenuation prov... stapedius reflex at 0 5 kHz as described ... (1968) It was measured as the ... corresponding contralateral st... curves obtained after recovery and ... lysis

The three Bell's palsy subjects ...



aring thresholds of one subject with fixed (affected ear) Right side Ear with normal stapedius
lekesy audiometry at 6 0 kHz without masking reflex (non affected ear) Masking is defined as rise in
1 0 5 kHz monaural (ipsilateral) masking noise threshold (*M*) during presentation of the masking noise
levels Left side Ear with stapedius paralysis

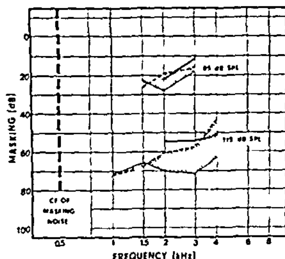
measurements were also lacking stape x upon ipsilateral 0 5 kHz pure tone on up to 130 dB SPL Their hearing were within 15 dB according to ISO (1964) between 0 125–8 0 kHz with udiometry The differences in hearing between the ears were less than 10 dB · drums were normal as shown by oto pic inspection with high magnification these subjects recovered from their · muscle paralysis (symmetry index 11 and then the masking measurements eated

ession was started with a determination unmasked threshold in one ear Then a noise was presented in the same ear hold tracing was continued for 0 5–2 until steady value was obtained Meas- s were performed in the affected and cted ear during paralysis as well as after

The measurements were performed in proof room with background noise below PL in the frequency range 315–8 000 Hz & Kjaer, tersfilter) In each session mask- investigated at one frequency at several evels in each ear At least 8 hours between sessions The hearing thresholds iced by means of a Bekesy audiometer (Grason-Stadler type E 800 with TDH 39 ear phones) with pulsating tone (2 5 pulses per sec) of fixed frequency, slow setting One of the subjects was investigated at the frequencies 1 0, 1 5, 2 0, 3 0 and 4 0 kHz and the two other subjects at 2 0, 3 0, 4 0, 6 0 and 8 0 kHz

The masking noise was produced by a Bruel & Kjaer noise generator (type 1024) Bandpass filtered noise centered at 0 5 kHz and a band width of 0 3 kHz (at 3 dB points) was used as the masker in all experiments The RMS level was varied from 85 to 120 dB SPL and the various intensities were presented in a randomised fashion The individual stapedius reflex thresh old for the masking noise was determined by measuring contralateral impedance change The ipsilateral reflex threshold for the masking noise was estimated on the basis of the subject's ipsilateral–contralateral difference for 0 5 kHz pure tone stimulation

Calibration of the earphones used for hearing threshold determination and for presentation of the masking noise was carried out in a 6 cc coupler and a Bruel & Kjaer condenser micro- phone type 4132 The determination of sensitivity of the stapedius reflex was measurements of the sound eardrum by the



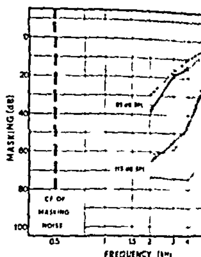


Fig 3 Masking as a function of test frequency in 2 subjects (left and right graph respectively) at two masking noise levels (85 and 115 dB) ——, affected ear during stapedius paralysis ——, the same ear a… ------, non affected ear Dotted areas … in masking during paralysis

RESULTS

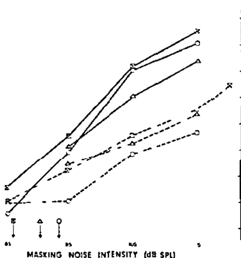
A *Determination of masking*

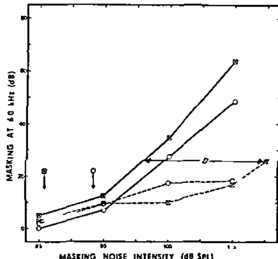
During the presentation of narrow band noise centered at 0 5 kHz, thresholds for pure tones in a wide frequency range were elevated both in ears with normal stapedius reflex and in ears with a stapedius paralysis Fig 2 shows a typical set of recordings of hearing thresholds obtained t 6 0 kHz without masking noise (−) and at ious levels of ipsilateral 0 5 kHz noise (85–115 B SPL) Left graph shows measurements in the ear with stapedius paralysis (affected ear) and right graph in the ear with normal stapedius reflex (non affected ear) Though thresholds increase with increase in noise level in both ears, the magnitude of the elevation in threshold is much greater in the affected than in the non-affected ear After the end of the noise presentation the hearing thresholds rapidly returned to the preexposure level The magnitude of masking produced by a certain noise is defined as the difference in hearing threshold at a certain test frequency with and without masking noise (e g *M* of Fig 2)

B *Masking as a function of test frequency*

Fig 3 shows masking produced by the 0 5 kHz noise in 2 subjects with unilateral Bell s palsy during stapedius paralysis and af… Two levels of the masking noise are … one (85 dB SPL) below the reflex th… the other one (115 dB SPL) well above … threshold The thin continuous lines … masking in the affected ear during … Heavy continuous lines show meas… the same ear after recovery of the … function Broken lines indicate mask… non-affected ear Shaded areas show … in masking between the affected a… affected ears during stapedius paraly…

It is seen in Fig 3 that masking … with intensity below reflex thresh… SPL) is independent of function of … muscle At high masking noise level … SPL) there is considerably more mask… with paralysed stapedius muscle th… with a functioning stapedius reflex … ences (shaded areas) are further… frequency dependent There is no … masking at the frequencies 1 0 and 1 5 … graph) Above 2 0 kHz there is a … masking that increases with frequen… graph) and reaches maximum at … dB) The masking in the ear w… reflex produced by the 115 dB noise … constant value of 70 dB in the freq… investigated (2 0 8 0 kHz) and it …





Masking at 4 0 kHz (left graph, 3 subjects) and 6 0 right graph, 2 subjects) as a function of the level 0 5 kHz masking noise ——, affected ear during s paralysis, -----, non affected ear Arrows with symbols show estimated ipsilateral stapedius reflex thresholds for the masking noise *D* indicates shift of the curves caused by functioning stapedius reflex

tend beyond 8 0 kHz After recovery (heavy masking in the affected ear equals that in -affected ear also at 115 dB

the third subject the stapedius reflex did not recover within 9 months according the above mentioned criterion In the stage of unilateral stapedius muscle paramasking was measured up to 8 0 kHz The s were similar to those shown in Fig 3, graph This subject also showed the most ounced difference in masking at the highest requencies The maximal difference was 54 t 8 0 kHz and 115 dB SPL intensity of the Hz *masking noise*

the non affected ear all differences between values of the two masking urement occasions were less than 6 dB age 2 7 dB) This was interpreted as a good bility of the method

Masking as a function of the masker level

magnitude of masking at 4 0 and 6 0 kHz function of the intensity of the masker in 3 is shown in Fig 4 The continuous lines masking in the affected ear during stapeparalysis and the broken lines masking in affected ear The arrows indicate the individual stapedius reflex thresholds for the masking noise estimated as described above It is seen that masking is equal in both ears below the reflex threshold When the masker level is raised above reflex threshold there is a rapid increase of masking in the affected ear The corresponding increase is considerably slower in the non-affected ear Thus reflex activity shifts the masking curve towards higher intensity and changes its slope The shift can be measured as the distance *D* of Fig 4 (right) This value represents the decrease in the 0 5 kHz masking noise level that is needed to obtain masking in *the ear with paralysed stapedius muscle equal to* that in the non-affected ear

D *Is antimasking due only to reflex attenuation of masking noise?*

Fig 5 shows a comparison between the attenuation at 0 5 kHz provided by the normal stapedius reflex and the shift of the masking curves (represented e g by *D* of Fig 4) The interrupted line is the attenuation determined as described by Borg (1968) It is based on stimulus response curves of the reflex measured as change in the ear's acoustic impedance in 19 subjects Attenuation is 7 dB per 10 dB increase of the

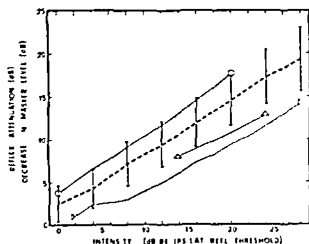


Fig 5 Dashed line shows mean and S D of the attenuation provided by the stapedius reflex measured on 19 Bell s palsy subjects Abscissa of the interrupted line stimulus intensity above ipsilateral reflex threshold for 0 5 kHz pure tone Continuous lines show average decrease in masker level that is necessary to obtain the same masking at the frequencies 3 0 4 0, 6 0 and 8 0 kHz in the affected ear as in the normal ear Abscissa of continuous lines masker intensity above estimated individual stapedius reflex threshold for the 0 5 kHz masking noise Each continuous line represents one subject Same symbols as in Fig 4

stimulus intensity This means that a raise of sound pressure in the auditory canal by 10 dB gives an increase of excitation in the cochlea by , 3 dB

The solid lines represent the average shift of masking above 2 0 kHz (the *D*-values) in each of the 3 subjects investigated

It is seen from Fig 5 that the continuous lines are close to or below the interrupted line The good agreement between the curves points strongly to the attenuation of sound transmission as the sole explanation for the antimasking effect provided by the stapedius reflex

DISCUSSION

The results from the present study showed that the low frequency noise masked high frequency sounds to a much greater extent in an ear without stapedius reflex than it did in an ear with normal stapedius reflex The decrease of masking due to stapedius activity thus improved hearing in a wide frequency range up to at least 8 0 kHz, probably even to the upper frequency hearing A normal stapedius reflex ... masked threshold up to about 50 dB ... masking effect can wholly be explained ... the attenuation of the low frequency ... provided by the stapedius reflex (Fig ...

It might seem surprising that ... frequencies below about 2.0 kHz ... unaffected by the stapedius activity ... Two reasons for this finding can be ...

(1) In the frequency range below 2... stapedius reflex attenuates both the ... the masked sound Thus a stapedius con... traction is followed by an approximate ... decrease in the transmission of both the ... and the masked tone through the middle ...

(2) Since masking close to the masker frequency grows slowly as the masker is increased (see e g data by Bilger & Hirsh ... Ehmer, 1959*a*) attenuation of the masker ... affect masking less in this frequency ... at greater distance from the masker

Functional significance of antimasking

The traditional view of the stapedius ... that of an attenuator of transmission ... frequency sound in the middle ear The ... tion amounts to about 20 dB (see Fig ... agrees with earlier results (Borg 19... finding of this study consists of the ... hearing at high frequencies The reflex ... tion of a low frequency masking ... hearing at high frequencies by up to a...

It has recently been shown (Borg & Zakrisson, 1973) that discrimination of speech ... significantly impaired in ears with ... paralysis at levels above the ... threshold The antimasking effect ... present study offers a probable explanation ... this finding the strong low frequency ... of speech sounds do not mask the ... high frequency ones in the presence of ... reflex The properties of a function... reflex and speech characteristics ... ally well adapted The significance of ... masking effect on speech discrim... has been suggested earlier on the ...

ients who had undergone surgery for oto- (Liden et al, 1964)

main conclusion of the present study is contraction of the stapedius muscle influ- s the whole frequency range of hearing Thus ccomplished by its selective attenuation of frequency sounds whereby their masking of frequency sounds is strongly reduced

ZUSAMMENFASSUNG

drei Patienten mit einseitiger totaler peripherer d uslahmung wurde die Grosse der Verdeckung n niederfrequentes Schmalbandrauschen bestimmt rsuchspersonen hatten normales Gehor im ganzen ereich bis 8 kHz. Die Horschwellenbestimmungen en mit pulsierendem Ton bei jeweils konstanter uenz im Bereich von 1 0 bis 8 0 kHz durchgefuhrt Verdeckungsgerausch diente ein kontinuierliches chen von 300 Hz Bandbreite mit einer Mittenfre z von 500 Hz und einer Intensitat innerhalb des ches von 85 b s 120 dB SPL. Die Messungen wurden hl an dem Ohr mit Stapediuslahmung wie an dem mit normalem Stapediusreflex durchgefuhrt und wahrend des Stadiums der akuten Lahmung und dem die Stapediusfunktion wieder normal geworden Unterhalb der Reflexschwelle war die Grosse der in beiden Ohren die gleiche Oberhalb der elle war sie dagegen in dem Ohr mit Stape g durchgehend grosser Der Unterschied war fur 6 und 8 kHz und konnte dort bis zu 50 ragen Nach der Ruckkehr der Stapediusfunktion e Grosse der Verdeckung in beiden Ohren die e Der Effekt der Verminderung der Verdeckung den Stapediusreflex konnte quantitativ aus seinem ungseinfluss auf das niederfrequente Verdeckungs sch erklart werden Es ist daraus zu schliessen dass tapediusreflex auf Grund seiner Minderung der stärkere niederfrequente Frequenzanteile bewirkten ng eine wesentliche Bedeutung fur das Horen von n Frequenzen hat

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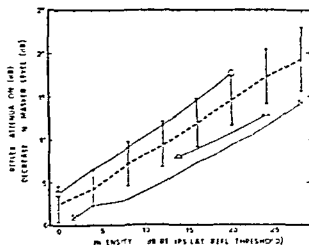


Fig 5 Dashed line shows mean and S.D of the attenuation provided by the stapedius reflex measured on 19 Bell's palsy subjects. Abscissa of the interrupted line stimulus intensity above ipsilateral reflex threshold for 0.5 kHz pure tone. Continuous lines show average decrease in masker level that is necessary to obtain the same masking at the frequencies 3.0 4.0 6.0 and 8.0 kHz in the affected ear as in the normal ear Abscissa of continuous lines masker intensity above estimated individual stapedius reflex threshold for the 0.5 kHz masking noise. Each continuous line represents one subject. Same symbols as in Fig. 4

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(1) In the frequency range be[illegible] stapedius reflex attenuates both the m[illegible] the masked sound Thus a stapedius [illegible] traction is followed by an approx[illegible] decrease in the transmission of both th[illegible] and the masked tone through the mid[illegible]

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animal was then prepared for electrophysio-cal recording a platinum iridium ball trode was placed on the round window and d with dental cement

esions were made at a point approximately ½ turn from the round window in the basal The bony wall of the cochlea was thinned a small diamond mill The finely thinned e was then removed carefully in pieces with licate pick, attempting to avoid damage to periotic labyrinth Final lesions of the endo-l membrane and cochlear vasculature were e with a fine microdissecting knife or an rode insulated except for an exposed tip approximately 5 microns in diameter) used uterize individual vessels Most lesions were ed in the scala media, two were restricted he scala vestibuli and one to the scala pani

trophysiological procedures

ear speculum coupled the external auditory tus to an earspeaker (PDR 600) A ½-inch lenser microphone with calibrated probe was used to monitor the sound pressure 2 mm lateral to the tympanic membrane oughout the electrophysiological procedures, bulla remained open

easurements of the cochlear microphonic) and N_1 response were made following ini-preparation of the animal These responses evaluated immediately following lesion at various time intervals following lesion ement The one microvolt isopotential our for the CM was evaluated with a eral Radio wave analyzer Frequencies ined included 0 08, 0 20, 0 50, 1 0, 2 0, 6 0, 10 0, 20 0, and 30 0 kHz The input ut (dynamic range) functions of the CM examined for 6 0 kHz, except for Animal 1 in which this function was examined at kHz The N_1 action potential was recorded series of 10 clicks presented at 10 per nd The clicks were generated by 2 V, 0 01 square wave pulses presented through the R 600 earspeaker Both latency and amplitude ie N_1 response were evaluated

Morphological procedures

Following termination of the electrophysiological portion of the experiment, the vascular system of the animal was perfused with Prussian blue solution (See Axelsson, 1968, 1972, for details of this procedure) The temporal bones were then removed and cochlea fixed and stained (Millonig's buffered osmic acid) The cochleae were decalcified in 10 % EDTA buffered to 7 4 Following washing, dehydration, and transfer to glycerol, the temporal bones were dissected with the aid of a dissecting microscope Sections of the external wall, spiral lamina, and basilar membrane were mounted on slides and the vasculature, sensory neuroepithelium, and supporting structures were examined under light and phase contrast microscopy The details of this new "soft-surface preparation technique" will be published elsewhere (Axelsson et al, 1973)

RESULTS

Only clear changes in electrophysiological responsiveness and histopathological observations following surgery are reported Omission of comment indicates that electrophysiological characteristics were unchanged following lesion and morphological features were characteristic of the normal guinea pig

Animal No 1 (19)

Surgery Restricted scala media lesion Little leakage of perilymph

Electrophysiology A fairly broad depression of the one microvolt CM contour was seen (Fig 1) Frequencies from 0 50 through 20 0 kHz were affected by this lesion, higher and lower frequencies were normal Immediately postoperatively, a maximum depression of 12 dB was seen at 4 0 kHz N_1 and input-output functions were affected in a similar manner to that seen in Animal No 2—see Fig 12

Morphology was not available on this subject

Animal No 2 (20)

Surgery Restricted scala media lesion leakage of perilymph

Fig 1 Change in 1 μV sensitivity contour following lesion restricted to scala media The post lesion response of each animal (——) are compared with their pre-lesion level of response (—)

Electrophysiology See Fig 1 Greatest effect is on frequencies in the 6 0 kHz range Maximum sensitivity change was 13 dB immediately after the lesion It was stable during the 3 hours of observation Fig 2 shows dynamic input output functions at 6 0 kHz Shift in input output function is consistent with a sensorineural loss The function was shifted to the right and the point of nonlinearity occurred at a lower intensity above 1 μV threshold Changes in N_1 potential are consistent with changes in the CM

Fig 2 Median and range of dynamic input-output function of the cochlear microphonic for three guinea pigs prior to lesion (control) and following scala media (GP 2 and 3) and scala tympani lesion (GP 6)

SVS

VSSP

AVAS

VLEM

CVL

Fig 3 Animal No 2. Discrete lesion of the ... cient vascular contrast injection ... arterioles (RAL) arteriovenous anastomoses ... the venules at the basilar membrane ... collecting venules (CVL)

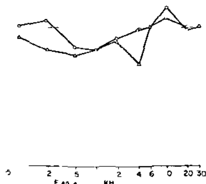
Morphology Damage was ... narrow segment of vessels ... vascularis and the vessel of the spiral ... and affected only peripheral parts of ... ing arterioles in scala vestibuli ... anastomoses in scala media and ... in scala tympani (Fig 3) Blood and ... found in the external spiral sulcus (F...

Animal No 3 (21)

Surgery Small penetrating les... media

f An mal No 2 In the outer sp ral sulcus between essel of the sp ral prom nence (VSSP) and the hment of the bas lar membrane (*arrow*) there is an accumulat on of blood and debris *VLBM* the venules at the bas lar membrane

ectroph) siology Restricted frequency ge of the cochlear microphon c contour 1) immediately following surgery Domi effect is in 2 0 kHz range (19 dB depress on)



Change n 1 μV sens tiv ty contour following les on cted to scala vest bul The post les on responses ch an mal (——) are compared to the pre les on of response ( )

During the following hour sensitivity decreased further for low frequencies The function then remained stable during the subsequent 4 hours Fig 2 shows dynam c input output funct ons at 6 0 kHz Changes were consistent with a sensorineural loss Function was to the right and the point of non linearity occurred at a lower intensity above the 1 μV threshold Changes in N_1 potential were consistent with changes seen in CM

Morphology Damage affected the stria vascu laris which was penetrated in its most basal part in a very restricted area Spiral prominence was also severed in a limited area whereas external spiral sulcus was unaffected

Animal No 4 (23)

Surgery Isolated scala vestibuli lesion with section of three radiating arterioles

Electroph) siology The 1 μV isopotential

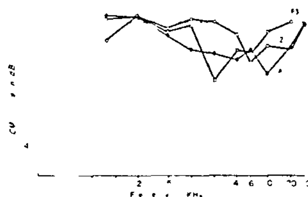


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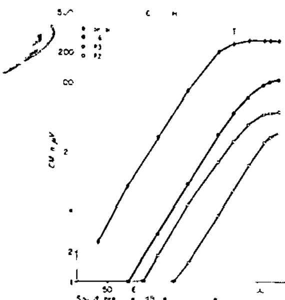


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SVS

VSSP

AVAS

VLBM

CVL

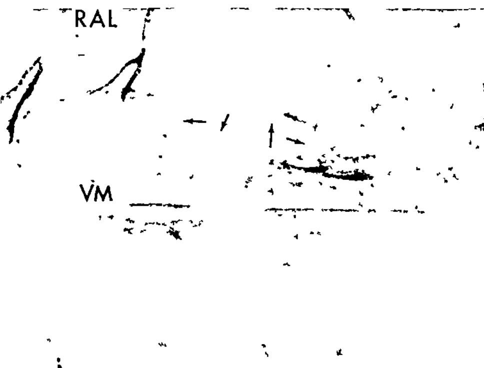
Fig 3 Animal No 2 Discrete lesion of the ... ment of the basal turn only influe... to the stria vascularis (SVS) and the vessel of ... prominence (VSSP) There is a narrow ... cient vascular contrast injection ... arterioles (RAL) arteriovenous ana... the venules at the basilar membrane ... collecting venules (CVL)

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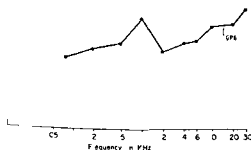
Animal No 3 (21)

Surgery Small penetrating les... media



Animal No 5 The scala vestibuli lesion (*arrows*) ompanied by a segment of uninjected vasculature ing all vessels peripheral to the lesion and the ing arterioles (*RAL*) central to the lesion The attachments of vestibular (*VM*) and the basilar (*BM*) membrane are unusually prominent probably due to accumulation of blood *SVS* stria vascularis

our is shown in Fig 5 The greatest effect at 4 0 kHz (13 dB) with an additional broad it in the low frequency range with a maxi at 0 50 kHz This occurred immediately



Change in 1 μV sensitivity contour following lesion cted to scala tympani

following the lesion and remained stable for the following 4 hours A continuously decreasing change in the N_1 response occurred during the 4 hours observation post lesion Immediately following the lesion, the N_1 amplitude decreased from 148 μV to 104 μV, then continuously decreased to 45 μV

Morphology Lesion is restricted to the scala vestibuli Three radiating arterioles are severed (Fig 6) Fracture lines eminate from the operated region in both directions to the scala media Attachments of Reissner's membrane both in spiral limbus and external wall were more prominent than normal, probably due to collection of blood corpuscles Radiating arterioles at site of lesion were uninjected both centrally and peripherally There is a segment of uninjected vessels corresponding to the lesion in the scala vestibuli, media and tympani

VRW



1 Change in 1μV cochlear microphonic sensitivity ur at intervals during staged surgery and lesion of dia See text

ial No 5 (26)

ery Restricted lesion of the scala vestibuli section of three radiating arterioles

ectrophysiology Fig 5 shows the 1 μV tential contour A maximum deficit at 1 0 was seen following the lesion

orphology Lesion is restricted to scala buli with sectioning of three radiating oles (Fig 7) A narrow segment of vessels sponding to the lesion is uninjected by ast in scala vestibuli, media and tympani 8) There is moderate hemorrhage on the of the cochlea at the site of lesion The ment of the basilar membrane in the nal wall appeared darkly stained

al No 6 (29)

ry Isolated scala tympani lesion with n of the vein of the round window - perilymph leakage Marked bleeding seconds

ctrophysiology Fig 9 shows a marked

9 Animal No 6 (*A*) Small lesion (*arrows*) only ng the vein of the round window (*VRW*) in the part of scala tympani close to the round window contrast is found outside of the vessel lumen (*B*) me lesion at high magnification showing the hole bone of the scala tympani and the contrast d vein of the round window

and relatively broad low frequency effect in cochlear sensitivity occurred following lesion The change in input output (Fig 2) and N_1 change was consistent with a sensorineural hearing loss

Morphology Lesion is confined to the basal parts of the scala tympani and to the region of the vein of the round window The vein, however, is injected by contrast (Fig 10A, B) No blood and little if any contrast was observed outside the vessel lumen

Further Electrophysiological Analyses

In an attempt to ascertain the amount of trauma caused to the cochlea by the surgical procedures, measures of the electrophysiological response were made at various times during the surgical procedure Figs 11 and 12 illustrate differences observed in the CM The 1 μV CM was little affected by the thinning, drilling and opening of the small fenestra however, following widening of the fenestra the CM decreased in sensitivity Fig 12 on the other hand shows a moderate decrease in the linearity of the CM before the fenestra was widened Moreover, there is truncation of the CM after widening and sealing This indicates the greater sensitivity of the input-output function to cochlear changes

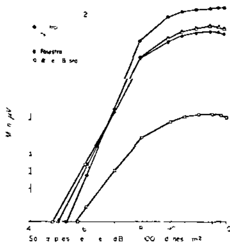


Fig 12 Change in input-output function of cochlear microphonic at intervals during staged surgery and lesion of scala media See text.

DISCUSSION

Reservations must be placed upon any conclusions drawn from this study Of primary concern is the small sample size of subjects and observations upon which the results of this paper are based In addition, both the electrophysiological as well as the morphological techniques employed have certain limitations However, we suggest that an approach using both electrophysiological and morphological techniques may permit a better evaluation of the normal or pathological cochlea than either technique alone The injection of Prussian blue in the cochlear vasculature provides information on the functional state of the vessels following these gross lesions

In the literature no similar experiments comparing morphological and electrophysiological effects of limited lesions to the cochlear are available Certainly, many different modes of influencing the cochlea in order to resemble clinical conditions with sensorineural hearing losses have been attempted Only a few of these have been confined and performed on the external wall (Stahle et al, 1972, Sugar et al, 1972 Stahle & Sugar, 1973, Axelsson & Hallen, 1973) These studies did not evaluate the functional state of the cochlea Tasaki et al (1952) examined the electrophysiological effects of lesions placed in the various scalae of the guinea pig They saw no change under any condition except following the introduction of foreign material into the scala media sufficient to press upon the basilar membrane Their recordings were made via differential electrodes introduced to the scalae of the guinea pig cochlea The discrepancy between the cochlear microphonic findings of that study and the present investigation may in part be based upon differences in recording procedures No morphological examination of the cochleae was made by Tasaki et al (1952)

Certain findings were clear in the present study In a number of cases in which CMs were recorded at various stages while the cochlear wall was thinned, picked and f... we saw little or no effect on the ... response (Fig 11) In general the ... factor on activity recorded from the ... window appears to be the presence of ... following an opening into any of ... scalae regardless of location or size of ... A correlation did exist between the ... lesion and the extent of reduction in the ... microphonic It appeared as though ... media lesion (3 cases) are ... specific in terms of the frequency ... was affected than scala vestibuli ... cases) We suggest that this observation ... that the primary influence of these ... not only hydrodynamic in nature ... may be a local change in mechanics ... the basilar membrane or a local ... change induced by the affected vascula...

The mechanical lesions had ... term influence on the organ of Corti ... by light- and phase contrast microscopy ... No changes were seen in the sensory ... supporting structures or vessels of the ... lamina In the short time studied in the ... experiment, the only morphological ... were those mechanically induced at the ... the lesion

The most interesting finding in the ... material is the effect of the variation ... and location of the lesion in the d... and on the different vessels on cochlear ... siveness In the two lesions affecting ... vestibuli, three large radiating arterioles ... severed The segment of the vascu... pheral to the lesion was uninjected ... in all three scalae Further, the ... ing arterioles were also uninjected ... centrally to the lesion from the ... is consistent with the previous ... (Axelsson & Hallén, 1973) in which ... "reflex closure" of the radiating ... seen We suggest that such a mecha... serve to limit effects of hemorrhage ... injured cochlear structures and ... Moreover, the segmental ... lesions may account for the ...



ˀ Animal No 4 Sensory and supporting structures basilar membrane immediately opposite lesion external wall No morphological changes observed (400 tim°s) *OHC 1–3* first, second and third row outer hair cells *IHC* – inner hair cells

relatively restricted electrophysiological ˌ observed

like some lesions (e g of the round low), the cochlear response was not de throughout all frequencies examined ıe scala tympani lesion, the vein of the round low was severed by surgery This vein re ˌ ı ost of the collecting venules in the scala in the most basal part of the cochlea risingly, in spite of the considerable initial le bleeding, the subsequent injected contrast ᵈ within the vessel lumen This may indicate during the 4-hour electrophysiological rvation period following lesion some process rred either in the vessel wall or soft tissues nding it to functionally ' seal ' this vessel pronounced electrophysiological changes be attributed to the proximity of the lesion ıe recording site It can also be assumed the size of the lesion has caused a consider leakage of perilymph Otherwise, it is g that this restricted vascular lesion with remaining vessel continuity on the venous side beyond the capillary areas should cause such a pronounced effect

Again from vascular considerations, it is surprising that the very superficial or deeper, but in all cases very limited vascular lesions, in the scala media were reflected in such severe electrophysiological changes We suggest this can only be explained on the basis of local mechanical effects—such as hydrostatic changes or distortion of the soft tissues of the scala media

In conclusion, the present investigation demonstrates a combined approach of evaluating the effects of mechanically induced lesions with the aid of electrophysiological and morphological measurements of both CM, N_1, and evaluation of vessels and sensory and supporting structures of the cochlea Our findings indicate that these restricted lesions result in restricted changes in the electrophysiological and morphological state of the cochlea This is encouraging

in that similar to the findings of the previous anatomical study it appears as though these types of restricted damage apparently are restricted both initially (functionally and structurally) and at longer times (structurally) in their effects We did not find a clear, orderly and detailed correlation between size and location of lesion and the change in cochlear activity Ongoing experiments both in animals assessed soon after surgery and more long standing experiments are needed to determine to what extent hydrodynamic changes segmental vascular injuries and other processes influence the electrophysiological and morphological state of the cochlea

ZUSAMMENFASSUNG

Mechanisch wurde die aussere Wand der Cochlea des Meerschweinchens geringfugig beschädigt Man untersuchte die Wirkung dieser Schaden auf Blutgefasse Haarzellen und das tragende Gewebe elektrophysiologisch und mittels histopathologischer Technik Diese Methode erscheint vielversprechend Die begrenzten Schaden resultierten in einer begrenzten Veranderung der Electrophysiologie sowie der Morphologie der Cochlea Haufige und regelmässige Veränderungen von Grosse und Lage des Schadens und der cochlearen Aktivitat wurden nicht gefunden In diesem Kurz Zeit Experiment sind keine morphologischen Effekte am Cortischen Organ beobachtet worden Der Schaden begrenzte s schmales Gebiet der ausseren Wand un er Be der dortigen Blutgefasse

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POSTNATAL DEVELOPMENT OF ENDOCOCHLEAR POTENTIAL AND STRIA VASCULARIS IN THE CAT

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act The postnatal development of the stria vascu and endocochlear potential was studied in the Maturation of the stria was completed at about 5th postnatal day The development of the basal was characterized by its reduction in size and shape by reduction of content of glycogen particles The nediate and marginal cells developed into complex nts with a large number of cytoplasmic compart containing a striking conglomeration of mito-lria Postnatal development of EP paralleled that of tria vascularis The increase in EP s values from (about 8 8 mV) to the 27-29th day (about 75 7 mV) bed an S-shaped curve

cochlear endolymph of mammalian species its an electrical polarization (endocochlear ial) of 80-90 mV positive with respect to lymph of scalae vestibuli and tympani y, 1952) The ionic composition of the lymph is unique, K$^+$ and Na$^+$ concentra approach that of intracellular fluids while concentration in the perilymph resemble of extracellular fluids (Smith et al, 1954) processes underlying generation of the ial (EP) and maintenance of rolytes in the labyrinthine fluids are still ly understood Experimental evidence in-es that EP is dependent on the metabolic ity of the stria vascularis and that it is not arily related to the ionic composition of inthine fluids (Davis et al, 1955, Tasaki, Konishi et al, 1966) Developmental have advanced our understanding of the ion between EP, stria vascularis and ionic composition of labyrinthine fluids Relevant data on postnatal maturation of labyrinthine fluids and EP have been reported by Bosher & Warren (1971) In the white Wister rat, the ionic concentration of K , Na$^+$, and Cl , in both endolymph and perilymph, reached near adult values at about the eighth postnatal day, while EP remained below +20 mV until the eleventh day They found also that maturation of the *stria vascularis and organ of Corti, excepting* the Claudius and Boettcher cells, paralleled that of the labyrinthine fluids Thereafter, EP increased rapidly, reaching adult value (+92 mV) at about the sixteenth postnatal day They hypothesized that the rapid development of EP between the eleventh and sixteenth postnatal day results from "a rapid increase in the electrical resistance of some portion of the mem branes bounding the endolymphatic space at this time' They also mentioned the alternative interpretation that EP development may be associated with maturation of its generator Schmidt & Fernandez (1963) and Änggård (1965) have related the development of EP to the postnatal maturation of the stria vascularis, an opinion supported by the work of Kikuchi & Hilding (1966) Their investigation showed, for the mouse, a progressive ultrastructural growth of the marginal, intermediate and basal cells of stria vascularis from birth to the eleventh postnatal day

In order to investigate further the relation between postnatal development of EP and stria

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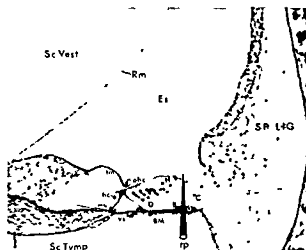


Fig 1 Cochlear duct of the first turn of an adult cat showing the position of the micropipette (*rp*) for recording resting potentials *Sc Vest*, Scala vestibuli, *Sc Tymp*, Scala tympany, *Es*, Scala media

vascularis, an experiment was designed in which EP was measured in kittens of known age and their stria vascularis examined with both light and electron microscopy The cat was selected as the experimental animal because this species has been and is extensively used in auditory physiology, while few data are available on the functional and morphological development of their cochlea (Rose et al, 1957, Pujol & Marty, 1970, Romand, 1971)

MATERIALS AND METHODS

Observations were made on 188 kittens and 24 adult cats All kittens were born in our animal quarters and their ages, ranging from three hours after birth to 142 days old, were known at the time of EP measurement Animals weighing less than 100 g during the first postnatal day were considered premature and discarded Each kitten was assigned at birth to a group of animals to be tested at a specific age, but 38 out of 188 animals either died before or during testing or were discarded after failure to measure EP

For recording EP the animal was anesthetized with sodium pentobarbital (30 mg/kg) given intraperitoneally After tracheotomy and fixation of the head, the round window was by opening the mastoid process in the formed by the lambdoid ridge and the border of the external auditory meat though the basilar membrane can be and punctured through the round window membrane, we decided to remove it in order to a clear visualization of the basilar memb should be mentioned that the removal round window membrane results in le perilymph and the question has been whether this procedure may alter the ge of DC resting potentials in both Corti's and the scala media There is no evide this is the case, since our measurements of others working with round window (Butler, 1965) or a large opening in scala (Tasaki et al, 1954) are about the same tude as that obtained by those work round window closed (Fernandez et al, Lawrence, 1967) By means of this posure, the recording micropipette inserted under high magnification with turbing the circulation of the spiral which in the kitten are large relative to the adult cat The recording electrode pe the basal turn between the spiral ve spiral ligament where the epithelium scala media is formed by the Hensen's cel more laterally by the Claudius' cells or the Boettcher's cells (Fig 1) H studies, however, revealed that some p tions were also made through De sensory cells (see Fig 13)

Micropipettes filled with 3M KCl or NaCl (impedance 5–8 megs) were used cording DC potentials The micropip mounted on a hydraulic microman which permitted reposition and placem electrode's tip at any desired locati basilar membrane An Ag AgCl electrod the micropipette to a Kithley ele (Model 250) The micropipette was a slowly through the basilar membrane organ of Corti and then into the scala The DC potentials of the organ of Co scala media were read directly on the

r and permanent records were obtained by ng the output of the electrometer into an Dynograph In some animals measure were made in one ear only while in others neasurements were bilateral

ty three kittens—representing ages from o 90 days old—were killed after completion P measurement by intravital perfusion of enhain s Susa fixative and their temporal s were removed and processed for histo al studies with light microscopy For on microscopy studies 26 kittens of ages ng from three hours to 30 days old were The cochlea was fixed intravitally and the mens prepared for electron microscopy es as described elsewhere (Hinojosa 1971)



Fg 2 Po...
(A) Prema...
15 days ...

RESULTS

stological studies

ostnatal development of Corti s organ Some res of the postnatal development of Corti s n are depicted in Fig 2 A premature kitten 2 A) shows a striking configuration of densely packed cells lining the inner s This embryonic structure referred to by (1863) as the Kolliker s organ de- and disappears progressively from to apex in about 25 days after birth

e postnatal development of the epithelium s over the pectinate zone of the basilar brane of the first turn was stud ed in detail it was through this area that the micro- te for recording EP was advanced into media In the newborn kitten the Deiters Hensen s cells are columnar shaped Their its are about 10 μ and 17 μ respectively 2 B) These cells reach maturation in 20–25 (Fig 2 F) Lateral to the Hensen s cells the ar membrane of the basal turn of the new kitten is lined by Boettcher s cells As n in Figs 2 A 2 E the Boettcher s cells—posed between Hensen s and Claudius —are in contact with the endolymph The d us cells are apparently located in the nal sulcus although some may be inter d between Hensen s and Boettcher s cells

At birth the ... a large nu... at the basal ... in contact ... villi and a ... (Fig 4) Th... plasmalem... apical (Fig 4 ... in some are... basal plasm... membrane a... than 20 da... by Ish yama ... about 20 d... grow rap... (Figs 2 ... Boettcher ... location b... 2 H) The ... show little ... in height) ... cells can ... underneat... of the m... cytoplasm... Claud us ...

Fig. 3. Ultrastructure of Boetcher's cells in a one day old kitten. Their junctional complex, lateral and basal plasmalemmae are illustrated in Figs 4, 5, and 6 respectively. ×5 814.

[illegible] The com- [illegible] adherens [illegible]

Fig 5 Middle portion of the lateral plasm[illegible] intercellular space in this area measured 25 Å. *Fig. 6* Basal area of the lateral plasmale[illegible] numerous infoldings The intercellular spa[illegible] about 160 Å ×21 450

opment, the cells of Hensen, Claudius and ther exhibit no cell multiplication or cell leration

spiral vessel of the basilar membrane a striking developmental feature con- g of a rapid reduction of its cross-sectional from about 500–600 μ^2 at birth (Fig 2 B) out 15–30 μ^2 within the next 10 days (Fig The large areas of the vessel may be related etabolic requirements of the developing s organ as mentioned by Lawrence (1971) ells lining the tympanic side of the basilar orane form a thick layer in the premature newborn kitten (Figs 2 A, 2 B), but within veeks the cells are considerably reduced in er (Fig 2 E)

Postnatal development of stria vascularis

tria vascularis of the newborn kitten (Fig 7) s considerably from the stria of a 30-day- r adult animal (Fig 12) The basal cells of ewborn are large and cuboidal with a pale ılar cytoplasm containing a few organelles d marginally near the cells walls and dant glycogen particles (Fig 8) These les were identified using the criteria of (1963) and Revel (1964), that is, in sections d with lead hydroxide, with or without yl salts, the glycogen appears as round or l particles of clear cut margins and high ty In the basal cells the particles are of the oparticulate type (about 300 Å in diameter) aining a substructure (30–40 Å) with dense (Fig 8, inset) Ribosomes are smaller cles (about 150 Å) and stain darker than gen (Fig 8) The nucleus of basal cells is and irregular in shape In the kitten and cat as well the basal cells may be found posed between marginal cells and cells of hment zone of the Reissner s membrane size shape and ultrastructure of the cells ing the attachment zone of the Reissner s ibrane of the kitten are similar to those ribed in the adult by Hinojosa & Rodriguez ındia (1966) The postnatal development of basal cells is characterized by a progressive ning (Figs 7, 10–12) and a diminution of ogen particles (Fig 9) so that in the 25–30 day old kitten, the cells exhibit the laminar feature of the matured cell with a large nucleus forming a striking prominence (Fig 12)

The postnatal development of marginal cells is illustrated in Figs 7, 10, 11 and 12 At birth, the apical plasmalemma in contact with endolymph exhibit microvilli, coated vesicles and a fully developed junctional complex (Fig 7) In some micrographs, however, the junctional com plex shows a gap at the level of the zonula occludens The basal plasmalemma presents shallow infolding which increases considerably in size and number during the first 25 postnatal days (Figs 10–12) The cytoplasm contains numerous vesicles and other organelles generally found in mature cells In the course of postnatal development the cells increase in size from about 14 μ in height to about 24 μ by the 30th day and to 37 μ in the adult A striking development feature is the accumulation of numerous mitochondria in the interdigitations of the basal plasmalemma

The intermediate cells of the newborn kitten exhibit a moderate number of organelles which gives to their cytoplasm a light appearance in contrast to the even lighter aspect of the basal cells and heavy density of the marginal cells The most noticeable developmental feature of inter mediate cells was a considerable increase in the number of interdigitations of their plasmalemma (Figs 7, 10–12)

In the kitten no basal lamina was ever found between basal cells and spiral ligament or underneath the marginal cells The absence of basal lamina in this location holds also for the adult cat (Kikuchi & Hilding 1966, Hinojosa & Rodriguez-Echandia, 1966) In the newborn mouse, however, Kikuchi & Hilding (1966) found a basement membrane underneath the marginal cells They assumed that this feature strongly supported the opinion that the marginal cells are of ectodermal origin (Engstrom et al , 1955 Weibel 1957) As in the adult, the strial capillaries of the newborn kitten are surrounded by an endothelial basal lamina which is con tinuous with that of capillaries of the spiral ligament (Fig 10) The endothelial basal lamina of

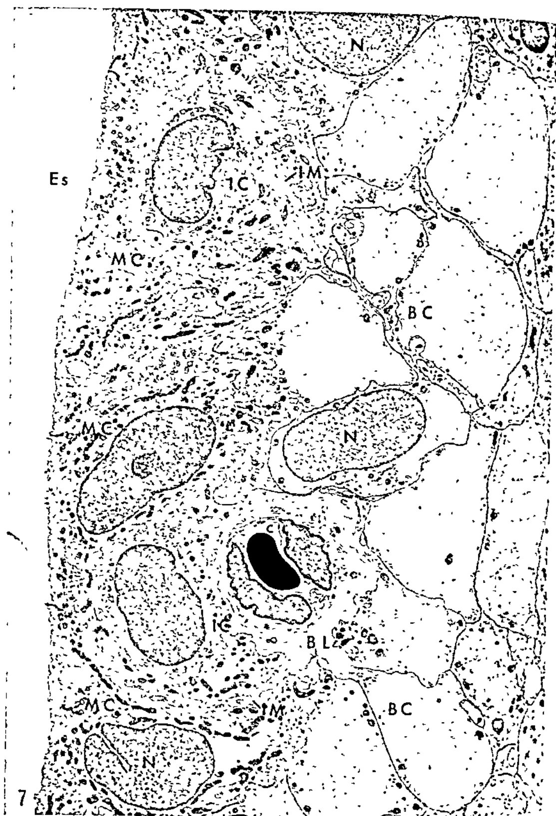
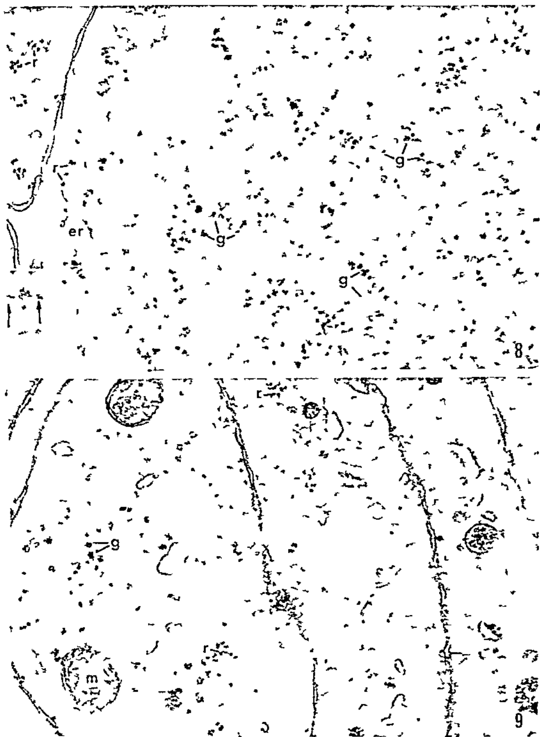


Fig 7. Electron micrograph of the stria vascularis (Basal turn) of a kitten three hours after birth The marginal cells (*MC*) show a few [illegible] the basal cytoplasm. The intermediate cells (*IC*) show a pale cytoplasm with numerous organelles. [illegible] digitations with the marginal cells can be seen. [illegible] cells (*BC*) are large, their cytoplasm appears [illegible] granular; it contains a few organelles located [illegible] periphery ×4 195.



' Basal cells of the str a vascularis of a newborn contain ng abundant glycogen particles (g) *Inset* 'gen part cles at h gh magnification showing the lar substructure (arrows) *er* Endoplasm c reti ' *r* R bosomes. 47 65? inset ×85 272

F'g 9 Basal cells of the stria vascularis of a 30 day old k tten showing few glycogen part cles (g) *m* Mitochondria *r* Ribosomes ×47 652.



Fig. 10. Ultrastructure of the stria vascularis in a 10 day old kitten. The most prominent change up to this age was the reduction in size of the basal cells (BC) and increase in density of their cytoplasm. This electron micrograph illustrates the extension of the basal lamina [illegible] the blood vessels (c). Arrow points toward [illegible] of the basal cells between the marginal cells.



1 Ultrastructure of the stria vascularis in a 20 day
itten The maturation of the stria are close to that
ited by an adult animal × 4 377



Fig 12 Stria vascular s of a 30 day old kitten The ultra structure is s m lar to that of the adult cat 3 556

ɛitten also exhibits expansions penetrating ⊦en the strial cells (Figs 7, 10-12, *IM*)

'hysiological studies

serial sections of the cochlea showed that puncture for recording DC potentials was ed at 4-5 mm from the basal end of Corti's ı In some specimens the puncture was made ,e level of Boettcher's cells while in others micropipette penetrated the basilar mem- at the level of Deiters cells (Fig 13) The ration of Corti's organ at any of these was associated with the recording of a ive DC voltage which changed polarity the electrode entered the endolymphatic (Fig 14) The negative potential of Corti's ı as a function of the animal's age is pre- d in Fig 15 There was a considerable tion on the measurements, but at birth ive DC voltages of about the same value as of the adult cat were obtained (Fig 14) variation among measurements may reflect rs such as speed of penetration, cell damage physiological conditions of the animal The potential of Corti's organ could be held for ort time before waning to zero, further ncement of the micropipette frequently reg- d a negative DC voltage again or a positive ı The latter indicated that the electrode trated the endolymphatic space Withdrawal ie micropipette or a second penetration ıgh the same path failed to register the ive potential of Corti's organ, but it could ecorded again by puncturing the basilar brane at a fraction of a millimeter away ı the previous hole On the other hand, the cochlear potential could be recorded for periods without decay and repetitively ture after puncture through the same path e data on the endocochlear potential as a ion of age are presented in Fig 16 The ly growth from birth to about the 27-29th natal day was a striking feature The value at birth (within 24 hours) was 8 8 ± 4 3 mV n ± S D , $N = 10$) and at the 27-29th post- day its value was 75 7 ± 8 3 mV ($n = 11$) growth of EP from birth to maturation ex-

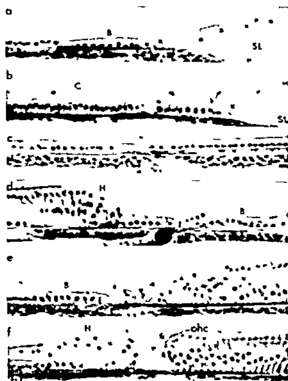


Fig 13 Samples of micropipette s paths The age of the kittens of *a* through *f* were 6, 28, 3 8, 9 and 25 days old and the negative dc polarization was 97, 80, 54, 80, 81 and 80 mV, respectively

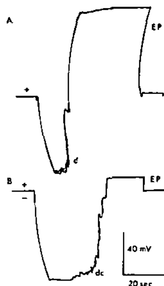


Fig 14 Cochlear DC potentials of an adult cat (A) and a newborn kitten (B) *dc*, Negative potential recorded in Corti s organ After waning the electrode was advanced into scala media registering the endocochlear potential (*EP*)

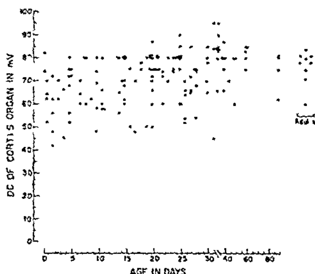


Fig. 15. The negative resting potential of Corti's organ as function of age. Each point represents maximum value obtained per one ear (N = 161).

hibited a rate of increase of about 1.7 mV per day up to the 9th postnatal day. Then the rate increased to 3.5 mV per day until the 20th, followed by a slow rate of about 1.8 mV per day until maturation. In the adult cat the value of EP was 80.4 ± 7.77 mV (n = 24).

DISCUSSION

Postnatal development of Claudius' and Boettcher's cells. The function of the Boettcher's cells is unknown although ultrastructural (I... et al., 1970) and histochemical (K... Plester, 1962, Rauch, 1964, Ishii & B... studies suggest that the cells may be p... with absorptive and/or secretory funct... opinion receives support from the fa... during development their apical plasm... in contact with the endolymph are provid... microvilli and a junctional complex.

An important developmental aspect ... Claudius' and Boettcher's cells is the ... maturation lag that of other cells in t... organ. Similar observations have been m... the rat (Bosher & Warren, 1971) and ... (Änggård, 1965). This developmental f... raises the question whether the ... Boettcher's cells represent an area of hi... trical resistance as postulated by Bosh... Warren (1971) to account for the grad... opment of EP. In our opinion, this is ... because the cells exhibit at birth a ... potential of the same magnitude as that i... adult, suggesting that the boundaries ... either mature or immature cells might ha... resistance. The solution of this problem r... measurements of the resistance across C... organ in the kitten.

Postnatal development of the stria vas... The maturation of Corti's organ and stria ... laris proceeds orderly from base to apex ... and other species (Bast & Anson 1949, Än... 1965, Kikuchi & Hilding, 1966, Bosh... Warren, 1971) although Larsell et al. (19... ported that in the opossum, the ... proceeds from the upper basal and lowe... turn in a basal and apical direction.

At birth the marginal, intermedia... cells of the stria vascularis of the cat ... ture but sharply differentiated from the ... ligament. All changed in size and sha... they reached maturation at about 25 da... birth. One striking developmental feat... stria vascularis is the progressive cha... "immature basal cell" in size, shape and ... content. The functional significance of ... changes is not clear. The intermedi... marginal cells develop into compl...

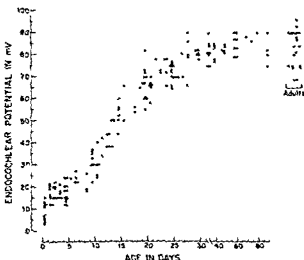


Fig. 16. The endocochlear potential as function of age. Each point represents maximum value obtained per one ear (N = 239).

a large number of cytoplasmic compart -s containing a large conglomeration of -hondria These morphological features -st that the structure may be involved in transport of fluids and ions That the stria major route of active ion transport has been ated by biochemical methods (Matschinsky halmann, 1970) and that the stria plays an rtant role in the maintenance of ion con- ation in the endolymph has been em- zed in the past The relation between ration of the stria and ionic composition of nthine fluids, however, has been worked -nly in the rat by Bosher & Warren (1971) his species the chemical maturation of nthine fluids parallels the histological ration of the stria vascularis, tectorial mem- and Corti s organ Maturation occurs at the 11th postnatal day, although Claudius' Boettcher's cells lag their maturation for seven additional days

e negative potential of Corti's organ Our vations support the opinion that it re- nts the negative intracellular potential on to all cells (Bekesy, 1952, Tasaki et al, Davis 1957, Dallos, 1968) The elec microscope provides convincing data that juncture through the area occupied by cher's, Deiters, Claudius' and Hensen's should record the intracellular potential of ll or cells found in the path of the micro e Their impalement seems unavoidable se the intercellular space between them is order of around 160 Å Although these are immature at birth, the records demon- that their membrane potential was fully oped

tnatal development of endocochlear potential postnatal development of EP in the cat ved the general pattern found in the opos- Schmidt & Fernández, 1963), rabbit (Äng- 1965) and rat (Bosher & Warren, 1971) rate of growth, however, varies from one s to another In the rat and mouse (Bosher arren, 1971, Schmidt & Fernandez, 1963), rowth occurs between the 10th and 15th atal day while in the opossum, rabbit and cat the EP grows to maturation beginning with the first postnatal day

Regarding the mechanism underlying matura- tion of EP, Bosher & Warren (1971) proposed that it may be related to the development of low electrical resistance of some portion of the mem- branes bounding the cochlear duct It is tempting to consider the Boettcher's cells as one of these areas since they are in contact with the endo- lymph for about four weeks Furthermore, their development and that of Claudius' cells parallels the development of EP, but it is not clear whether these events are related

The opinion that the development of EP is associated with the maturation of the stria vascularis is supported by the observations of Schmidt & Fernandez (1963), Änggård (1965), Bosher & Warren (1971) and our observations Tasaki & Spyropoulus (1959) located the generator of EP in the stria vascularis and the striking changes occurring in this structure dur- ing postnatal development strongly indicate that the progressive increase in magnitude of EP up to the 27 days old kitten is related to maturation of its generator in the stria

ZUSAMMENFASSUNG

Die Entwicklung der Stria Vascularis und des endokoch learen Potentials nach der Geburt, wurde bei jungen Katzen untersucht Die Stria war ungefahr am 25 Tag nach der Geburt ausgereift Die Entwicklung der Grund zellen wurde dadurch ausgezeichnet, dass sich die grossen Zellen mit reichlichem Zellplasma in Grosse und Form zu kleinen abgeflachten Zellen verringerten Die mitt leren und ausseren Zellen entwickeln sich zu komplexen Bestandteilen mit zahlreichen Zellplasmaabteilungen, die eine beachtliche Anhäufung von Mitochondria ent halten Die Resultate zeigen eine Parallelentwicklung zwischen dem endokochlearen Potentials und der Stria Vascularis nach der Geburt Die Zunahme der endo- kochlearen Potentialwerte von der Geburt (ca. 8 8 Milli volt) bis zum 27 bis 29 (ca 75 7 Millivolt) beschreibt eine S formige Kurve

LIST OF ABBREVIATIONS

BC, Basal cell
BL, Basal lamina
BM, Basilar membrane
B, Boettcher s cell
C, Claudius cell

D, Deiters cell
Es, Endolymphatic space
H, Hensen's cell
IC, Intermediate cell
ihc, Inner hair cell
IM, Interstitial membrane
MC, Marginal cell
N, Nucleus
ohc, Outer hair cell
p, Pericyte
Rm, Reissner's membrane
SL, Spiral lamina
SP LIG, Spiral ligament
SV, Stria vascularis
tm, Tectorial membrane
vs, Spiral vessel

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COMPARISON OF COCHLEAR MICROPHONIC POTENTIALS FROM ALBINO AND PIGMENTED GUINEA PIGS

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ct Recent behavioral tests would seem to indicate uditory thresholds of albino guinea pigs are sig itly lower than the thresholds of pigmented guinea In the present study, cochlear microphonic 10μV ential (CM sensitivity) functions were recorded he round windows of these two groups of anesthe guinea pigs (*Cavia procellus*) as one measure of ner ear performance For 15 of 20 tested frequencies een 70 and 7 000 Hz) the mean CM sensitivities lbino animals were not significantly different from ean CM sensitivities of 6 pigmented animals The mum cochlear potential magnitudes obtainable at frequencies (100 1 000 and 4 000 Hz) from each l group also were found statistically alike It is ided that there is no difference between the tested ı pig strains with regard to the measured parameters of cochlear microphonic potential

ea pigs have been commonly used labora subjects for almost all aspects of auditory rch Most of these animals undoubtedly of the albino variety, but it is conceivable some investigators have studied mixtures he albino and pigmented types without ng the fact in the subsequent reports Thus, reader cannot be certain which variety was when the guinea pig type was not specified point could be important were the be oral auditory threshold measurements of (1973) to be confirmed In that study the o guinea pig's hearing threshold was pared with the pigmented guinea pig's threshold At all frequencies tested, Crifò reported that the albino animals displayed significantly better hearing (lower thresholds) than the pigmented group

The behavioral testing scheme which was used in the Crifo study was first reported by Anderson & Wedenberg (1965) and later used by Ernstson (1972) The method, simply stated, was the conditioned suppression of shivering due to cold An alteration or interruption of a regular shivering pattern could be induced when a tone, followed by a shock, was presented to the animal Shivering appears to be a good choice for a behavioral parameter because it may not require learning by the guinea pig, whereas learned tasks, such as the water licking described by Heffner et al (1971), can require long training periods

However well shiver behavior compares to other behavioral methods for auditory threshold testing, the results reported by Crifò (1973) indicate that it may not be valid to include both albino and pigmented strains together in studies of the hearing mechanism, e g in cochlear electrophysiological studies The present report addresses itself to an examination of the cochlear microphonic potential (CM) from the inner ear in order to determine if the CM sensitivity (isopotential) function or the maximum CM magnitude (indicators which may have a direct relationship to the functioning endorgan) differ between the two strains of guinea pig

work was supported by Public Health Service Grant

Table I *The range of the sound intensities required to produce 10 μV from each of the tw[...]*

Frequency, kHz		0 07	0 1	0 2	0 3	0 4	0 5	0 6
Albino data range dB re 10^{-1} N/m²	Max	− 2	− 4 7	− 6 8	−12 0	−13 7	−19 0	−19[illegible]
	Min	− 8 6	−13 7	−20 8	−20 6	−23.3	−27 0	[illegible]
Pigmented data range dB re 10^{-1} N/m²	Max	+ 1 8	− 2.3	− 9 5	−14 1	−21 2	−24 8	[illegible]
	Min	−10 7	−15 3	−19 0	−23 7	−26 2	−31 9	−3[illegible]

METHODS

A total of 12 guinea pigs (*Cavia procellus*) of both sexes, with body weights between 250 and 350 g were used in this study The animals were divided into two groups, the first containing 6 pigmented animals (from Hilltop Lab Animals, Scottdale, Pa , 15683) and the second having 6 albino animals (4 from an established colony at Kresge Hearing Research Institute, 2 from Camm Research Institute, Wayne, N J , 07470) All pigmented guinea pigs had dark colored eyes as well as patterned or solid colored fur Each of the 12 animals exhibited a good pinna (Preyer) reflex to sudden loud sounds

The animals were deeply anesthetized with allobarbital (100 mg/ml) and urethane (400 mg/ml) anesthetic administered intraperitoneally (0 8 cc/kg) Respiration was maintained artificially by means of a tracheal cannula and heart rate was monitored for the duration of the experiment A conventional postauricular surgical approach on the left side was used to expose the round window membrane of the cochlea After fastening the head in a head holder, the left auricle was removed, leaving a ring of cartilage at the meatus, to facilitate connection of a sound speculum

Sound intensity, in the closed field system, was determined with a calibrated probe tube microphone (Western Electric 640 AA) at a point approximately 3 mm from the tympanic membrane in the external meatus Cochle[...] phonic potentials (CM) picked up by [...] bead electrode on the round wi[...] amplified 1 000 × (Princeton Appl[...] CR-4 preamplifier) and the magnitud[...] fundamental frequency compone[...] measured on a wave analyzer (Hewle[...] 302A) Ten microvolts was chosen as [...] measurement level All experiments [...] ducted with the animal in a sound- booth (Industrial Acoustics Co Inc, [...]

RESULTS

CM sensitivity functions for the g[...] albino and the group of 6 pigmented g[...] are plotted together in Fig 1 The[...] represent the mean 10 μV isopotential [...] 20 frequencies for the two groups B[...] have the same general shape and f[...] frequencies they are nearly superimp[...] albino guinea pig sensitivity funct[...] circles), seems to exhibit a bias to[...] sensitivity compared to the pigme[...] pig sensitivity curve (closed circles) [...] for most frequencies the means of on[...] lie within the ranges of the other T[...] for each group and frequency a[...] Table I

A non parametric statistic, the Ma[...] U test, was used to test for the s[...] difference between the means of th[...]

Table II *For each of 20 frequencies, the probability (Pr) of the occurrence of the exper[...] (μ_1 and μ_2) are the same as determined by a two-tailed Mann Whitney U test*

Frequency (kHz)	0 07	0 1	0 2	0.3	0 4	0 5	0 6
$Pr\{H_0, \mu_1 = \mu_2\}$	0 396	0 818	1 00		0 009	[illegible]	03[illegible]

ps

l	09	10	1.5	20	2.5	30	35	40	50	60	70
0	−15 6	−17 5	19 0	−15 0	−10 5	− 5 0	− 3 7	− 7 9	− 4 7	− 8 0	12.0
5	−21 0	22.4	−21 0	−19 2	−15 1	−11 6	−13 4	15 7	16 3	−17 0	17 4
3	−19 5	−18 9	19 3	−18 9	−13 6	5 0	− 7 3	10 1	−10 1	−11 1	14 2
7	25 3	−30 4	27 0	−21 3	19 3	−16 9	−14 9	−22 0	20 0	18 5	19 9

ıch frequency Table II contains the results ıis test For 15 frequencies, the null hypos, that the albino and pigmented groups the same mean sensitivity, cannot be ted at the 5% significance level Only 5 of 0 differences (at frequencies 400, 500, 1 500,) and 7 000) are found significant at the evel

ıe second measure of auditory performance rted here is the maximum magnitude of the For each group of guinea pigs, Table II the mean of the maximum potential as as the range of the data at 3 frequencies 1 000 and 4 000 Hz) Results of the Mann tney U test on these maximums are also ded in Table III In all cases the null hypos cannot be rejected at the 5% significance

DISCUSSION

data indicate that, for the most part, albino pigmented guinea pigs are alike in terms of 10 μV CM sensitivity functions and the imum CM magnitude which can be gene- l Bias in the direction of less sensitivity in the albino data may well be due to one ıal which performed poorly Indeed, 10 of requencies from this animal represent the me low sensitivity end of the group range t 500 Hz where the separation between the es is greatest, the difference between the group means is 5 6 dB This value does not seem large when compared to the standard deviation, at 500 Hz, of 10 dB obtained by Lawrence & Yantis (1957) for 35 normal guinea pigs In that study, Lawrence and Yantis report standard deviations on a 1 μV sensitivity function which range from a minimum of about 7 dB to a maximum of about 16 dB However, it should be noted that a 10 μV CM sensitivity level may have smaller variance than the 1 μV CM level because of a greater signal to noise ratio, i e, relatively less uncorrelated physiological noise and possibly less contamination by acoustic nerve action potentials [1]

The above findings may be compared to the available behavioral data Crifo (1973) reports that the 2 guinea pig groups are quite different in terms of their auditory thresholds He observed a 23 3 to 33 dB difference between the animal strains with the pigmented animal being less sensitive (recall that the possible bias seen in the CM data suggests that the pigmented guinea pig is more sensitive) All frequencies which Crifo tested were significant at the 0 1% level

Relatively little research has been done concerning the relationship between the CM and the hearing threshold Wever (1959) has compiled correlatable behavioral CM data on five species, including the guinea pig, and concluded that the general shape of the CM and behavioral

[1] Weiss et al (1971) reported intensity nonlinearity possibly caused by neural contamination

given the null hypothesis (H_0) that the pigmented and albino guinea pig mean 10 μV sensitivities

09	10	15	20	25	30	35	40	50	60	70
0 064	0.240	0 042	0 004	0 180	>0 240	0 438	0 180	0 132	0 396	0 042

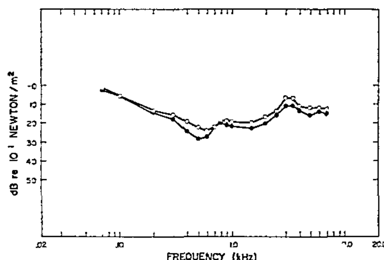


Fig 1 Mean sensitivity (10 µV) [illegible] curves for albino (○—) and (●—●) guinea pigs.

functions for a given species differ in three aspects, the low frequency slope, the point of maximum sensitivity, and the upper limit of response Additional reports have expanded the number of species tested and have confirmed an interspecies variability in both functions (e g , McGill, 1959, Dalland et al , 1967, Price, 1971) Part of the difference between the CM and behavioral functions can be accounted for by the very nature of the CM signal recording technique An electrode placed near the inner ear will tend to favor signals from the nearby sources of the spatial array Thus, the round [illegible]dow recording technique shows bias for [illegible]h audio frequency signals since the receptor of the first cochlear turn are closest to the electrode Discordance between the CM [illegible] behavioral functions may also be [illegible] from measurements of the behavioral [illegible] field (e g , closed or open field) When [illegible] siders the above points it is not [illegible] find that the shapes of the CM and [illegible] threshold functions are not alike

There is reason to believe that the [illegible] the shape of either the CM or the be[illegible] threshold function, induced by [illegible] manipulation, may be a useful measure [illegible] lear function Changes in CM after [illegible] overstimulation have been correlated [illegible] ear structural damage seen histologically [illegible] et al , 1944, Davis, 1953) Additional [illegible] hearing loss which results from acoustic [illegible] or ototoxic drugs also correlates with [illegible] findings (Suggit, 1943, Elliott & McGe[illegible] Stebbins et al , 1969) Experimental [illegible] which combine induced changes in CM [illegible] haviorally determined auditory thresholds [illegible] histology are lacking at this time The [illegible] findings imply the possibility that [illegible] a difference in behavioral auditory [illegible] could also be manifested by the [illegible] microphonic potential

In conclusion this study indicates [illegible] is little or no difference in the CM [illegible] functions and maximum CM magnitude [illegible] pigmented and albino guinea pigs. The [illegible] therefore, do not appear to support the [illegible] tions of Crifo (1973) that the two [illegible]

Table III *The mean and range of the greatest CM produced by a group of 6 albino and a group of 6 pigmented guinea pigs*

"Pr{ }" – The probability of the occurrence of the experimental samples given the null hypothesis (H_0) that the pigmented and albino guinea pig mean maximum CM voltages (μ_1 and μ_2) are the same as determined by a two-tailed Mann Whitney *U* test

Frequency (kHz)		0 01	1 0	4 0
Pigmented CM (µV)	Maximum	1 100	970	580
	Mean	875	785	456
	Minimum	700	600	350
Albino CM (µV)	Maximum	1 100	890	500
	Mean	667	715	382
	Minimum	450	490	260
$Pr\{H_0, \mu_1 = \mu_2\}$		0 31	0 588	0 094

cantly different hearing thresholds However, one must remain aware that electrophysio-l and behavioral methods are not neces- equivalent measures of auditory function ts obtained by each of the two methods conceivably reflect entirely different as- of the hearing process since, as mentioned , the two methods have not been experi- illy compared

ACKNOWLEDGEMENT

author wishes to thank Mr David Marques for his nce in collecting some of the data

ZUSAMMENFASSUNG

Verhaltensteste scheinen darauf hinzudeuten, dass rschwelle von Albino-Meerschweinchen signifikant er ist als bei pigmentierten Meerschweinchen In liegenden Untersuchung wurden cochleare mikro- che 10 μV Isopotentiale ("CM Empfindlichkeit") nden Fenster dieser 2 Gruppen am anesthasierten hweinchen (Cavia procellus) als ein Mass der ohrleistung gemessen Bei 15 von 20 untersuchten enzen (zwischen 70 und 7 000 Hz) war die durch liche CM Empfindlichkeit von 6 Albino-Tieren er durchschnittliche CM Empfindlichkeit der 6 ntierten Tiere nicht signifikant verschieden Die al erreichbare cochleare Potentialhohe bei 3 enzen (100, 1 000 und 4 000 Hz) bei den Gruppen lls statistisch ahnlich Wir schliessen daraus, ein Unterschied zwischen den untersuchten Meer- inchenstammen hinsichtlich der gemessenen Para- des cochlearen mikrophonischen Potentials t

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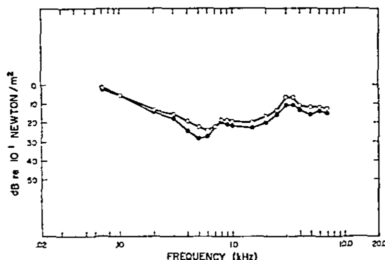


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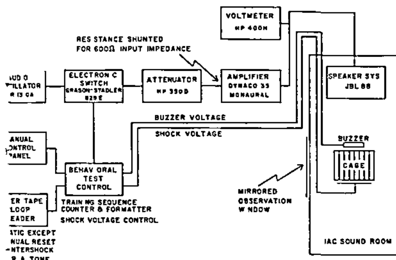


Fig 1 Diagram of testing apparatus

ıposure The exposure ıtself ıs more open ͜uestıon We have tried putting the sound tly to the tympanic membrane through a ılum This makes it possible to measure the ısity of the stimulus with a probe tube from microphone inside the orifice of the specu- A discrepancy may arise in that the animal, when anesthetized and clamped as securely ossible, may still achieve slight movements ı a slight movement may move the speculum quite different position and so a quite differ- intensity of sound may be delivered to the panic membrane We have also tried de- ing the sound from a speaker three inches ı the pinna, and aimed directly at the exter- orifice of the external auditory meatus, with probe tube just inside the orifice This gives nstant sound level, but we do not know isely what the sound level is at the drum ıbrane This seems to be the most consistent we can deliver the stimulus, therefore, de- the doubt as to what the level is at the panic membrane, it seems better to achieve consistency where it can be measured accu- ly

MATERIALS AND METHODS

study was conducted along classical lines t is, the work can be divided into the owing separate parts Behavioral training and determination of audiograms, exposure to pure tones, redetermination of the audiograms, sacrifice, and examination of the organ of Corti These represent distinctly separate disciplines which should be managed by experts in each area, thus the multiple authorship of this paper Dr Ades was in overall charge of the project and also participated especially in the anatomical work, Dr Kokko-Cunningham took part in the anatomical portion and had the special responsibility for the electron microscopy and histochemistry, Dr Trahiotis supervised the animal testing portion, and Dr Averbuch, who is an electrical engineer, supervised the exposure and the instrumentation

PROCEDURES

Apparatus

Five monaural chinchillas were trained in the apparatus which is diagrammed in Fig 1

The output from the audio oscillator was delivered to the electronic switch for gating the tone on and off The gated tone was adjusted in amplitude with an attenuator from a known value of speaker drive (measurements taken with a voltmeter for a continuous tone)

The behavioral test apparatus, which was built in this laboratory, contained the electronics necessary to program the sequences of stimuli and to operate the buzzer and shock device The

COMPARISON OF HEARING IN THE CHINCHILI

H W Ades[1] C Trahio

From the Departments of Electrical F
Univer

(

Abstract Behavioral anatomical and h effects of acoustic overstimulation produce tone were investigated In each of five mo chillas high frequency hearing loss was pro *was related to locus and extent of hair cell l* audiograms were fairly consistent with tho c quiet and the anatomical data on presence o hair cells Histochemical and electron micro nation of selected portions of the cochl revealed a series of changes in hair cells v may not influence their functional propert

Tones or noise of high intensity have for many years to stimulate the ears mental animals and thus to produce ear structures and changes in funct inner ear Periodically, developmen techniques for examining inner ear for measuring inner ear functio the closer and closer correlat physical characteristics of the stim ear pathology and inner ear functio

The best of the older studies emplo cal responses by inner ear elements or methods to obtain measures of audit They related inner ear function with t parameters of acoustic input, freque sity, and duration of sounds used However in very few of these studie damage and ear function been stud same animal after controlled exposur

The present study attempts to corr three components as well as is po

This study was carried out under grants fr Aeronautics and Space Administration N 14 005 074 and National Institutes of H Number 1 R01 NS09983-01

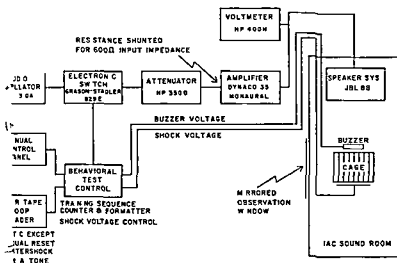


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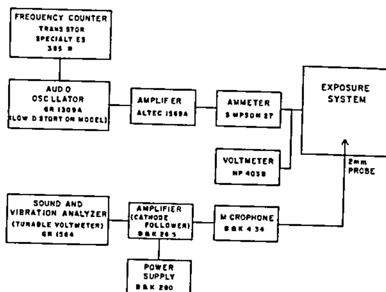


Fig 2 Diagram of expe

training sequences could be manually triggered from the control panel, or could be sequenced automatically by a paper-tape loop The paper tapes were computer generated and provided the desired randomization of intertrial interval

The sound pressure level (SPL) for a known value of speaker input voltage at one frequency was measured for all frequencies at 24 points in the training cage The reference SPL used at any frequency for threshold determinations was the average of these 24 readings Calibration in this manner allowed all SPL's at all frequencies to known with respect to a single, pre-set drive ting without the need for readjustment

Audiometry

The chinchillas were trained to avoid shock by crossing a barrier in the shuttle box (see Fig 1) in response to presentation of a 2-sec sample of a sinusoidal tone of 1000 Hz Shock was accompanied by sound from a buzzer mounted in the roof of the cage Thus, the sound of the buzzer acquired aversive value and could substitute for the shocks to keep the animals motivated in the months of future testing

When the animals had reached a criterion of not less than 90 % correct avoidance responses, the stimulus parameters were changed, and threshold determinations were begun using a method of descending limits The duration of test tones was 750 msec with a rise of 50 msec The stimulus consisted of bursts separated by 250 msec If moved from one end to the other of box at any time during the presenta three tone bursts, shock was not del a correct response was recorded T the stimulus was then attenuated by the test was repeated after a rand time interval (averaging about 20 sec) tern was repeated until the animal sponded The SPL was then increased the animal responded correctly, the reduced 10 dB and the sequence co the animal *failed* to respond to the crease in SPL, the procedure was d and the threshold was taken to be tween the latter level and the level duced the last positive response Ea was tested at 2, 4, 8, and 16 kHz, or 0 1 0 500 and 1 0 kHz each day Since the got better and better at detecting low uli, many measurements of "thres to be taken When these remained stable over at least 20 sessions at each a threshold value was calculated

Following completion of the tests animals were tested at three levels of using a low pass noise filtered at 10 was presented continuously at

of 0 dB, 10 dB, and 20 dB SPL Measure-s of levels of noise were made at 24 posi-in the test cage The sound field was quite rm Each animal was tested at least eight at each test frequency for each of the three of masking noise

sure to pure tones

of the animals was anesthetized and ex-l to presentations of a 4 kHz tone for one at 130 dB, 125 dB, or 120 dB SPL A block am of the apparatus is shown in Fig 2 specially constructed plastic chamber was for exposing chinchillas to sound levels › 140–145 dB SPL The maximum attain levels were dependent upon frequency, due ›onances in the rectangular exposure cham- The basic chamber was made of one-thick transparent plastic, 4 inches high, 4 s wide, and 13 inches long internally and open at the ends On one end, any of a va- of speaker fittings could be attached A · Altec 421A, 15-inch, low frequency er could be mounted, with an adapter, for encies below 500 Hz For the lower fre-cies heavy aluminum extensions could be to tune the length of the chamber to the ·d resonant frequency while the end of the iber, opposite the speaker, was sealed with luminum plate For higher frequencies, a of Altec 290 D speaker drivers were connected e chamber through a cast aluminum horn were driven in phase When necessary to ·e chamber resonance, an acoustic absorbing iber was attached on the end of the exposure iber opposite the speaker

·th the animal s head in the chamber, a 2 probe was inserted into the ear of interest, ·was brought as near as possible to the ear l opening The probe, attached and cali-ıd to a one half inch microphone, was left ace during the entire exposure In addition e SPL of the principle exposure frequency, irst few harmonics and subharmonics were ced with a tunable voltmeter to insure ist excessive distortion The absolute sound ı calibration was made with a B&K 4220 "Pistonphone" coupled to the one half inch microphone The probe was calibrated, over the frequency range of interest, against a second one-half inch microphone

Anatomical investigation

The surface preparation used for the initial phases of anatomical study was described in Stockwell et al (1969) It will not be repeated here The following histochemical methods were employed

The cochleas (with the exception of animal No 89) were fixed with 2% glutaraldehyde in 0 1 M cacodylate buffer of pH 7 2 The fixation was begun by diffusing the fixative gently through the fenestra ovalis and the fenestra rotundum, with a small outlet hole made in the apex It was continued by immersing in the same fixative for 6 hours During the latter period the bony walls of the cochlea were ground down and the coils of the organ of Corti were detached and removed from the modiolus The resultant segments were treated as free floating pieces thereafter When fixation had been completed, the segments were rinsed from 12 to 24 hours in 0 1 M cacodylate buffer (pH 7 2) which contained 0 2 M sucrose They were then incubated for acid phosphatase activity as described by the Barka-Anderson (1962) modification of the Gomori reaction (1952) Incubation time was 60 min at 37°C, after which the segments of the organ of Corti were rinsed again and then placed in 1% OsO_4 in cacodylate buffer for 1 hour Following another rinse in the buffer, the segments were mounted in glycerine under cover glasses They were then ready to be observed under the light microscope Animal No 89 was fixed directly in 1% OsO_4 buffered with veronal acetate and no incubation was done

After recording the presence, absence, or damage of hair cells of the organ of Corti (the resultant computer drawn cochleogram will be illustrated for each animal), the segments were removed from the slides, dehydrated, embedded in Epon–Araldite, and tissue from representative areas was thin sectioned In some specimens, only a narrow area was sampled for elec-

tron microscopy, while in others segments up to 1000 μm in length were sampled at 25 to 50 μm intervals Thin sections were stained with uranyl acetate and lead citrate and viewed in either the RCA EMU3H or the Siemens Elmiskop A1

RESULTS

Pre-exposure data

The mean thresholds of the 5 animals taken in quiet circumstances are shown in Fig 3 in solid line In addition, the similar curve for Miller's 36 animals is shown in broken line (Miller, 1970) This demonstrates a comparison with the results of at least one other investigator, and shows general agreement with work done on the same species at a different laboratory, with different apparatus The two differ significantly only at the highest frequencies, 8 kHz and 16 kHz This may be the result of some slight difference in the test chambers which is impossible to analyze

An indication of the variability of threshold measurements can be gained from Fig 4, in which representative data from 2 animals, Numbers 90 and 96, are plotted as a function of number of thresholds included in the determination of average threshold Standard deviation was y constant at about 5–10 dB in the last five urements Fig 5 shows the average thresh-

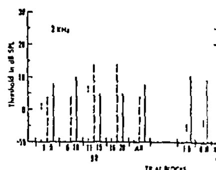


Fig 4 Samples of mean median, and stand of pre-exposure threshold responses of an and No 96 in blocks of five trials

olds of the 5 animals for four con pre-exposure testing i e, quiet and 0 20 dB levels of masking No data wer at 16 kHz in the presence of masking cause of the variability in the sound l the non uniform speaker response cluded adequate definition of the level the thresholds were systematically a the level of the masking The appar in sensitivity of the animals at 1 kHz the fact that the sound system had a point at that frequency

It is possible to compare the s masker level ratios necessary for de each test frequency to the data reported (1970, p 176) (from Miller J, unp servations) In order to make these co

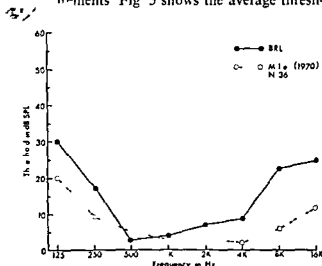


Fig 3 Average pre-exposure thresholds of the 5 animals included in this study (——) and of Miller s 36 animals done at Central Institute for the Deaf (   )

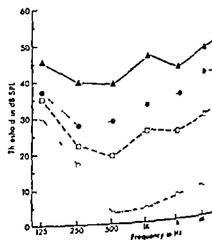
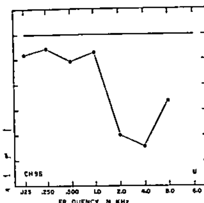
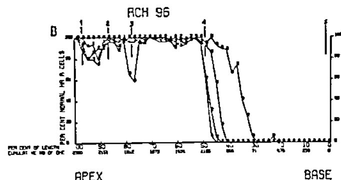


Fig 5 Pre-exposure thresholds in quiet (three levels of masking (□ = 0 dB level, ● ▲ = 20 dB level)





(A) Hearing loss animal No 96 following expo) 4 kHz, 130 dB for 1 hour (B) Cochleogram of No 96 (0 l ne – inner hair cells 1 2, 3 lines = rows of outer hair cells from mod olar s de out) Vertical lines and numbers indicating where samples were taken for EM

·tual SPL value of the noise spectrum level ured in the test cage was utilized The signal ectrum level ratios measured in the two s typically do not differ by more than 2 dB he test frequencies used in both studies result is interpreted as further evidence of lequacy of the sample and the testing pro es

exposure data

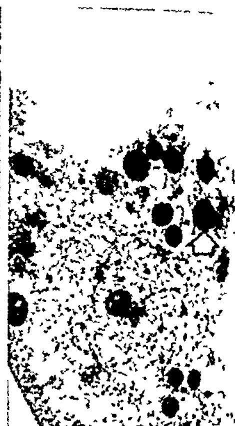
al No 96 Fig 6 is a composite of the data is animal Fig 6A is the curve of hearing ith normal hearing assumed to be a straight as is ordinarily done with human data in idiogram Loss of hearing for the several encies is represented as points below the ht line There is negligible hearing loss nt at the first four frequencies however are losses of 40 dB at 2 kHz 44 dB at 4 and 27 dB at 8 kHz Ostensibly, the loss at 4 kHz, but it will be remembered that andard deviation is roughly 5 dB so a 10 oss is probably significant but must be dered as marginally so

e computer drawn cochleogram (Fig 6B) s complete cell destruction from the base) 30 % of the length rising sharply from point indicating a virtually intact cochlea % of the length The inner hair cells appear about 75 % of them being present at 35 % of the length and 90 % or higher from 40 to 50 % of the length There is little difference between the rows of outer hair cells in this respect There is scattered cell loss at the apical end with only the third row of outer hair cells showing appreciable loss Samples were taken from the specimen for electron microscopy from five areas The areas are marked by vertical lines in Fig 6B

In the first three areas in addition to the missing cells other changes that were considered abnormal were seen Some fibers in the inner spiral bundle and nerve endings underneath the inner hair cells appeared swollen (Fig 7A) Outer hair cells in all three areas showed an accumulation of inclusion or multivesicular bodies under the cuticular plate (Fig 7B) These vesicles were surrounded by membranes and contained granular or fibrillar material and a few lipid droplets They varied in size from 0 5 to 2 μm The inclusion bodies occurred in moderately increased number as compared with the normal animals and some of them exhibited acid phosphatase activity Lamellar structures, somewhat resembling tightly packed lateral membranes were also seen under the cuticular plate in the outer hair cells of these areas

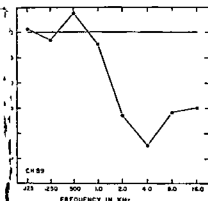
In area 4 many more of the multivesicular bodies appeared in the outer hair cells however the region in which the cells contained abnormal numbers of these was relatively narrow

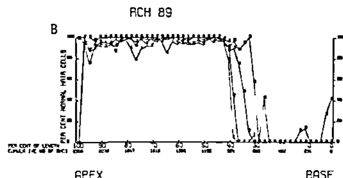

A




C







(A) Hearing loss animal No 89, following ex... to 4 kHz, 125 dB for 1 hour (B) Cochleogram ...mal No 89 (see legend for Fig 6B)

...nly 200 μm from the area of heavy damage ...is in contrast to animal No 93 (see below) ...h had a wider area of abnormal outer hair ...near the edge of heavy damage) In addition, ...fibers of the inner spiral bundle were ...n, and occasionally fused, or giant stereo-...on both inner and outer hair cells were ...ent Rods or crystalloids exhibiting a tubu-...bstructure were more numerous than those ...ly seen in inner hair cells of normal animals ...showed signs of degeneration in that they ...ined occasional lipid droplets (Fig 7C), ...sometimes showed acid phosphatase ac-

...ea 5, 400 μm from the base of the cochlea, ...sted only of a flat epithelium Cytoplasmic ...elles were scarce The cells of the limbus ...is were fewer than normal and quite ...iken while the Böttcher cells appeared ...normal

...imal No 89 This animal showed a hearing ...curve (Fig 8A) much like the preceding ...with loss peaking at 4 kHz The peak is at −45 dB The only other frequencies which show significant losses are 2, 8, and 16 kHz

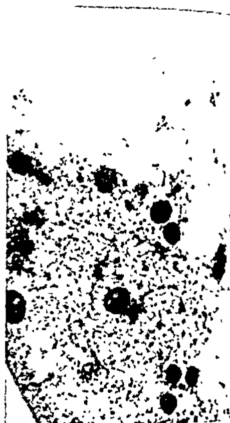
The cochleogram (Fig 8B) shows essentially a complete absence of hair cells (except for a minor number of inner hair cells) up to 30% from the basal end of the organ of Corti This is a fairly sharp-edged lesion with the inner hair cells again virtually completely present within 2%, i e, at about 32% of the length The outer hair cells are all present once more at about 43% of the length From that point on to the apex there is scattered, minor loss of outer cells and the inners remain at full count

Electron microscopy was done, but the ultrastructural preservation was inferior to the other 4 animals Consequently, about all that can be said affirmatively is that there was an abnormal accumulation of multivesiculated bodies in the outer hair cells near the major lesion Systematic sampling was not carried out

Animal No 97 This animal showed distinct loss of hearing at 0 250, 0 500, and 1 0 kHz, and a much greater loss at 2, 4, and 8 kHz, the

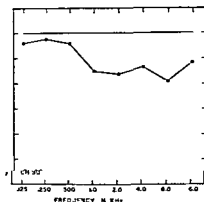
...(A) Inner spiral bundle from area 1 (middle of ...coil) of animal No 97 Some of the nerve fibers ...ollen (*arrow*) and have empty spaces Others are ...n and filled with fibrillar material × 11 000 (B) Outer ...ell 2 from area 4 (end of middle coil) of animal ...3 showing accumulation of multivesicular inclusion ...(arrow points to one) under the cuticular plate ...ion bodies are surrounded by a membrane and ...n fibrillar or granular material and lipid like droplets × 11 700 (C) Inner hair cell from area 4 (end of middle coil) of animal No 96 One of the rods with tubular sub-structure shows accumulation of lipid (*arrow*) × 6 300 (D) Outer hair cell 1 from area 6 (lower basal coil) of animal No 93 Multivesicular inclusion bodies have increased in number and fill the subcuticular cytoplasm. Some have lost their surrounding membranes and small lipid like droplets are dispersed in the cytoplasm × 11 200

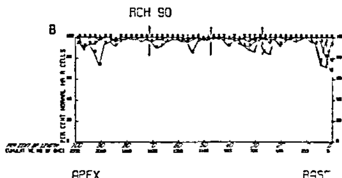

A











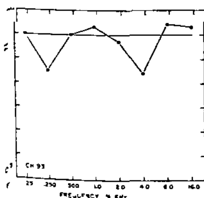
) (A) Hearing loss, animal No 90, following expo-) 4 kHz, 120 dB, for 1 hour (B) Cochleogram of l No 90 (see legend for Fig. 6B)

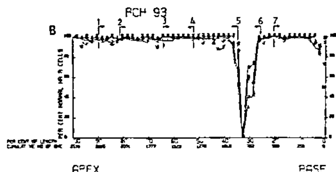
· lower three frequencies, 0 125, 0 250, and ·) kHz (Fig 10A) This statement is based .. e thesis that nothing below a 10 dB loss :d be counted as an actual loss

: cochleogram (Fig 10B) shows little dam- The greatest amount is just at the base ·ıp to 10 % of the length, the loss being about · in the three rows of outer hair cells The inder of the length shows only significant tions of one of the rows at a time, one · deviation near the apex showing 25 % ge to the third row of outer hair cells inner hair cell row is intact throughout ·les for electron microscopy were taken the areas indicated in Fig 10B

,art from the missing cells only a few ultra ,tural changes were interpreted as abnormal Outer hair cells in all areas sampled showed an accumulation of inclusion or multivesicular bodies under the cuticular plate There were occasional giant stereocilia in all the samples, more frequently on inner than on outer hair cells The nerve endings and supporting cells appeared normal

Animal No 93 This animal had apparently significant losses of hearing at 0 250 and 4 kHz, and normal hearing otherwise (Fig 11A) The loss at 4 kHz would fit the results on other animals, at least it would not be discordant The loss at 0 250 kHz, however, fails to match the results on the others In no other case are there losses at two test frequencies with an intervening interval of three test frequencies at which no loss occurs Whether this is to be "explained" on





l (A) Hearing loss, animal No 93, following expo- o 4 kHz, 125 dB, for 1 hour (B) Cochleogram of ·l No 93 (see legend for Fig. 6B)

the basis of some unknown error in testing or on the microscopic results remains to be seen The cochleogram (Fig 11 B) shows a complete loss of cells over a short distance at about 30–39 % of the length counting from the base This might account for the 4 kHz loss in hearing but there is no separate lesion to account for the loss at 0 250 kHz The two hearing losses are of about equal magnitude If the 4 kHz loss is to be accounted for as being all that would be expected from a narrow lesion in the basal turn, why is there no obvious lesion in the apical turn to account for the loss at 0 250 kHz? On the other hand, if the result on 0 250 kHz is to be accounted for on the basis of testing error, why is that at 4 kHz not equally spurious? The specimen was sampled at seven regions shown by vertical lines in Fig 11 B

The first four areas (counting from the apex) all showed swollen nerve endings under the inner hair cells and swollen fibers in the inner spiral bundle Outer hair cells showed an increase in the number of inclusion bodies under the cuticular plate (Fig 7B) Rods or crystalloids exhibiting degenerative changes were seen in inner hair cells, however, we are not prepared to say that the changes noted are related to the hearing loss at 250 Hz

In areas 5 and 6 the main lesion consisted of central area about 250 μm in width in which the acoustic papilla was absent and was represented only by flat cuboidal epithelium which was relatively empty of cellular organelles The hair cells were missing in an area about 250 μm wide in either direction but in these zones the supporting cells were present filling the space formerly occupied by hair cells, and maintaining the general contour of the organ of Corti There were acid phosphatase positive lysosomes and Golgi complexes in the supporting cells Cell debris from degenerated hair cells was rarely seen, and in these areas there were numerous nerve fibers which were quite normal in appear ance In the region from 750 1000 μm away from the maximum damage area the subcuticular portions of the outer hair cells were filled with multivesicular bodies (Fig 7D) which con

tained large numbers of lipid li- d- the area from 500–750 μm from t- damage were outer hair cells in wh- vesicular bodies not only occupied t- ular region, but filled the entire ce- the multivesicular bodies had lost t- rounding membranes and their co- sisting of lipid droplets were dispersed out the cytoplasm of the hair cells Th- numerous degenerated nerve end- outer hair cells in the area also The cells contained many tubular rods s- showing degenerative changes Gi- were relatively frequent

In area 7, the outer hair cells st- than the normal complement of m- bodies in the subcuticular region Un- hair cells there were many swollen ne- Inner hair cells also contained degen- with occasional acid phosphatase - merous giant stereocilia were seen

DISCUSSION

Many articles on the correlation be- ing loss and damage to parts of th- start with at least an implicit ass- the place of maximum vibration of th- membrane determines receptor repre- acoustic stimuli By a curious recipro- this 'place' theory of hearing th- explain the data while the data provide for a 'place' theory of hearing It- if one looks only at 4 kHz exposur- ment with a simple place theory is - but by no means perfect The prese- only to exposures of 4 kHz and - biased toward belief in a place th- should not be so considered and the - arguments should be held in abeyan- moment This paper is designed to - to the problem of correlation of - inner ear and damage to function

In that respect there are some - crepancies between hearing resul- pathology in animals Nos 89 9- cording to a rigorous place theory -

oss at 4 kHz should be much higher than it is not complete Likewise, hearing for the r frequencies should be as much impaired r 4 kHz if not more, inasmuch as the recep- were destroyed for the more basal portions e cochlea as well as the presumptive 4 kHz n In actual fact, however, a considerable int of hearing remains for those frequencies receptors are transducing the higher fre- cies?

fore attempting an answer to that question, s turn to the histological methods for a ent A source of error may be found in nanner of judging the condition of the cells the construction of the cochleogram alone is the object of electron microscopical and chemical investigation of the same speci- as are represented in the cochleograms will form the subject of a separate publi n, but some comment is warranted here in the immediate vicinity of the lesion 1 is shown on the cochleograms show vary- egrees of abnormality including ultrastruc- and histochemical changes It must be said the ultrastructural preservation left much desired It was originally thought necessary ount the specimens in glycerine to count for the construction of cochleograms We ute part of the inferior preservation to this ldition, the long rinses and incubation in an milieu after fixation also contribute to poor rvation Even so, the changes interpreted thological in the present material were not d in normal animals treated similarly

ost of the changes which we interpreted as rmal have been described earlier The ing of afferent nerve endings and fibers r the inner hair cells after acoustic trauma been reported by Spoendlin (1971) He dis d it as a reversible change Spoendlin (1970) rted similar swellings as a result of changes smotic pressure (like the fused stereocilia, this change is not specific to acoustic ma but is a general reaction to more than kind of noxious influence (Engström et al,) The survival time for our animals was ciently long to allow for the disappearance of all reversible changes, and, unlike Spoendlin, we interpret them as permanent, however, the number of swollen endings in our specimens apparently is less than those described by Spoendlin (1971), and might be presumed to be the irreversible residue of a larger number

Increase of lysosomes in the infracuticular region has been reported by other authors (e g, Engstrom et al, 1970, Ishii et al, 1968) after both acoustic trauma and treatment with oto- toxic antibiotics Ishii et al (1967) also reported an increase in lipofuscin pigment and acid phosphatase activity in the hair cells of elderly humans The inclusion bodies somewhat re- semble lipofuscin, however, they usually have numerous small rather than a single large lipid droplet The disappearance of the surrounding membrane, and the release of the lipid droplets into the cytoplasm have been illustrated by Spoendlin also (1970)

With survival time of 1–2 hours, Hensen bodies have been seen to increase in number and complexity after exposure to jet-engine noise (Engström & Ades, 1960) Several Hensen bodies were encountered in the hair cells of the present material, though no more than in normal ears The accumulation of membranous mate- rial, similar to the lateral membranes, which was particularly prominent in animal No 96, was also described by Spoendlin (1970) after acoustic trauma

Some of the changes are designated "degen- erative" These are degenerative changes on general grounds That is, they are the sort of changes that would indicate degeneration in any cell But how far does degeneration have to go to render the cell non-functional, or semi-func- tional, or functional intermittently? What does this do to threshold measurements? No answers are available immediately We are dealing with uncertainties and variables at both ends of the process, and no method is yet known which will resolve the difficulty

Perhaps the thresholds obtained in the pres- ence of masking noise *following* the creation of cochlear lesions may help to explain the small amounts of hearing loss reported One might

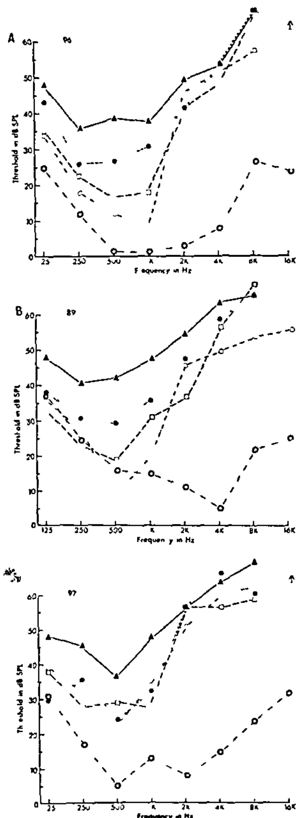


Fig 12 (A) Animal No 96 Curves showing thresholds obtained pre-exposure (solid circles with stars) post exposure thresholds obtained in quiet ( ) with 0 dB level masker ( ) with 10 dB masker (●) and with 20 dB masker (▲) (B) Animal No 89 (see legend for Fig 12A) (C) Animal No 97 (see legend for Fig 12A)

justifiably wonder how animals 96 and ... with some of the basilar membrane ... stripped of receptors, hear so well as ... indicated by data in Figs. 12A and ... introduction of masking noise affects ... ance not only at the lower frequencies ... 8 kHz These data may be interpreted ... that the animals were using receptors ... *middle* regions of the cochlea to transduce 8 kHz information Note the presence of microscopically healthy tissue in the ... regions of the cochlea and the attendant ... effects (Figs 6B and 8B)

Animal No 97 s data allow for a ... this interpretation Note that animal ... differed from 89 and No 96 in ... Animal No 97 (Fig 9B) had varying ... of inner hair cells present in the basal ... of the cochlea and a greater number of ... hair cells missing on the apical side of the ... One may speculate that the *inner* hair cells ... be mediating the behavioral responses ... on this animal (Fig 12C) Further ... predict that the masker effectiveness ... reduced at the lower test frequencies ... 0 500, 1 0 kHz) because of the ... losses in the region 50-80% of the ... from the base Since the animal ... efficiently transduce the lower level ... noises could not mask Indeed the data ... that this is the case When the masker ... sufficient to mask the lower frequencies ... also appears to be a masking effect ... Whether this indicates that the 8 kHz ... was processed by the animal ... middle portions of the cochlea by ... cells or in the basal regions by inner ... a difficult question to answer It seems ... data could be interpreted as indicating ... inner hair cells were transducing the ... at 8 kHz and were finally masked at the ... noise level The interpretation is based ... assumption that the outer hair cells ... to about 80% of the distance from the ...

ved, and, if used, would result in a greater
ng loss than that measured in the quiet
results on masking may actually provide
rt for the anatomical findings That is to
he remaining inner hair cells in animal
97, and the remaining outer hair cells in
al No 96, may be functional in whole or
rt despite the evidence of changes in them
changes, from one point of view, may be
and represent primarily metabolic change
in turn would tend to vindicate the surface
ration and, hence, the cochleograms
results of the present experiments are at
nce with those reported by Ward & Duvall
) There seems to be some doubt about
sting in that series of experiments (personal
munication) There is also doubt that their
mical analysis went far enough At least
ave never been convinced that positive
ts were forthcoming from the type of
ods that were used
milarly, these results have no special bearing
ose of Spoendlin (1972), who reported that
95% of the fibers emanating from the spiral
ion of Corti innervate inner hair cells,
the remaining 5% innervate outer hair
The 5% seems a remarkably small percent-
ssuming that the outer hair cells are related
ionally to loudness, however, there is no
sary reason to assume any exact relation
between loudness detection and number of
ons innervating the receptors Perhaps the
n the percentage seems small is our pre
ation with loudness in this series of experi-

his is not the precise, clear statement of facts
would like to present On the one hand the
g method is open to question On the other,
anatomical methods are imprecise Added
is is the suspicion that the subject of the
iments, the chinchilla, is an inadequate
al on which to base broad conclusions
t human hearing which is, after all one of
objects of research of this kind The chin
a is simultaneously more sensitive to noise
less sensitive to frequency differences than
human Further studies should be done on
monkeys which are apparently more closely
similar to humans in those respects

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ZUSAMMENFASSUNG

Die Wirkungen akustischer Überreizung auf anatomische, histochemische und Verhaltens Parameter wurden an funf einohrigen Chinchillas untersucht Beschallung erfolgte mit einem Dauerton von 4 kHz Die an Hand von Audiogrammen festgestellten Horausfalle stimmten ziemlich gut mit der Lokalisation sowie der Ausdehnung des Haarzellen untergangs im Cortischen Organ überein An Haarzellen ausserhalb der Hauptzerstorungsherde wurden ferner eine Reihe von histochemischen und ultra strukturellen Veranderungen festgestellt deren funktionelle Bedeutung noch unklar ist

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TEMPORARY THRESHOLD SHIFTS PRODUCED BY EXPOSURE TO VIBRATION, NOISE, AND VIBRATION-PLUS-NOISE

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ct Changes in auditory sensitivity were recorded Békésy audiometer technique following exposure ration in each of 300 cpm (5 Hz) and 1000 cpm Hz), to broad band noise of 82 dB SL and to Vibration at a frequency of 300 cpm and an ude of 6 mm, or at a frequency of 1000 cpm and iplitude of 3 mm, were applied until the subject no longer tolerate them (a 20 min period) by a controlled vibration exciter There was no signifi- change in threshold sensitivity after exposure to ion alone Exposure to vibration and noise simul isly caused greater threshold shifts (TTS) and longer ry time than exposure to noise alone This was ally true with 1000 cpm vibration It was suggested he effects of the combined noise and vibration be the results of some disturbances of physiologi- meostasis or possible mechanical interactions with od supply

is exposed simultaneously to multiple en- mental stresses of noise, vibration and factors Occupational deafness may be d or aggravated by the additive effects of al environmental factors, especially vibra- However, little work has been done so far ie effect of vibration on the auditory organ ie great bulk of the literature on the effect bration on auditory function deals mainly animal experiments (Popoff, 1932, Temkin, , Yamamoto, 1949, Seo, 1955, Esaki, M saki, Y, 1958, Nomi, 1958, Nakamura, , Jauhiainen, et al, 1969) In order to avoid y to humans, we must use experimental ials for most studies on mechanical injury, and some of the general conclusions drawn from animal experimentation can be applied to man However, the abdominal system of mice in the supine position has been found to have a natural frequency between 18 and 25 Hz whereas the natural frequency of the trunk of humans is known to be about 3 Hz (Goldman & v Gierke, 1961) Humans differ from animals not only in size but in physiological and anatomical structure and psychological behavior

Consequently, the results of animal experiments must be subjected to careful scrutiny before being applied to humans Unfortunately, we have no information on the effect of vibration on the human ear expect for the reports presented by Morita (1959) and Guignard & Coles (1965), as far as we know

Therefore, basic studies should be devised to evaluate the effect of each environmental factor alone and of all factors together in a controlled situation The purpose of these studies is to investigate the separate effects of noise and of vibration, and their combined effect, on human hearing

EXPERIMENTAL CONDITIONS AND METHODS

The experimental conditions of our study included variations in frequency, magnitude, intensity, mode of application, duration, and situation of the subject, so that the mechanical

nted in part before the 2nd Extraordinary Inter nal Audiology Congress (Tokyo), Oct 21 1965

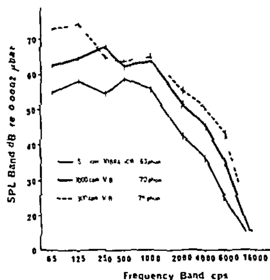


Fig 1

responses to vibration of the body could be determined The changes of threshold before and after these exposure conditions were evaluated by means of fixed Bekesy audiometry with a continuous tone at 4 kHz in subjects with normal hearing who had fully familiarized themselves with Bekesy audiometry

Subjects

Eight male members consisting of medical students and teachers in the Osaka City University [illegible]ok part in the test program Their range in age [illegible]s 27–35 years They had normal hearing and had had considerable listening experience on Békésy audiometry, i e they had had more than 5 practice sessions with this type of audiometry under approximately identical conditions on different days to prepare for the present experiments Hearing of their right ears only was tested

Vibration exciter

The vibration exciter utilized for this experiments was the Kishia Seizo AU type vibration testing machine, which is driven by centrifuged force It is capable of producing vertical and horizontal direction of sinusoidal vibration through a frequency range adjustable by 200 cpm up to 1 800 cpm (3 3–30 Hz) The frequency is expressed as units of cycle per [illegible] abbreviated cpm The maximum [illegible] the table is 5 mm, the maximum [illegible] 12 g at 1 800 cpm, and the maximum [illegible] weight of the test piece is 250 kg [illegible] were accomplished from a console [illegible] table control station

The noise produced by the vibra[illegible] can be a very disturbing factor in th[illegible] the level and the frequency of the [illegible] controlled by enclosing the excita[illegible] with a sound proof cover, which [illegible] the sound pressure level of higher [illegible] (above 1 000 Hz) as shown in Fi[illegible] *subjects also closed their ears [illegible]* protectors during the vibration [illegible] That is, since the noise produced by the [illegible] device was of a low level under 70 [illegible] experiments and since the subjects [illegible] earphones during vibration exposure, [illegible] of the vibratory device is not important [illegible] study

Frequency, amplitude and durat[illegible]n of [illegible] employed

Sinusoidal vibrations at frequencies of [illegible] and 1 000 cpm (5 Hz and 16 7 Hz) [illegible] the present study, because vibration [illegible] presented the greatest motion of t[illegible] frequency range for humans (Coerm[illegible] and the frequency distributions of [illegible]tions occurring in industrial environ[illegible] found to have the highest displa[illegible] vicinity of 1 000 cpm (Miura 1959)

All subjects were exposed to spec[illegible] tudes at each frequency, an ampl[illegible] at a frequency of 300 cpm and an [illegible] 3 0 mm at a frequency of 1 000 cpm [illegible] could not longer tolerate the vibra[illegible]tion of 20 min) This exposure time [illegible] was the longest time a subject c[illegible] maintaining the same posture [illegible] a shaking chair

Intensity level of noise employed

This was adjusted to produce a [illegible] at 82 dB of white noise from an ear[illegible]

), a 700 ml coupler) The earphone was
ily fixed to the right ear

tone and test room

pure tone at 4 000 Hg was selected in this y, as the reason that TTS producing by sure to white noise is the largest amount at) Hz (Ward, 1963) The subject's thresholds recorded with the Bekésy audiometer (Rion, del A-1014A) and an earphone (Iwasaki hin Model DR-305, 5 Ω, a 6 ml coupler) a continuous tone at 4 000 Hz The record-attenuator changed attenuation at the rate dB/sec, in steps of 2 dB If some of the cts tended to confuse the threshold tone tinnitus or were unsure of what they heard, threshold stimulation tone was at once re-ed with an interrupted tone adjusted to 10 interruptions per sec) and a rise-decay time 5 msec by means of the electronic switch n, Model SB 02) All of the threshold meas ments were carried out in a separate room near the vibration structure, which had d proof walls and was quiet enough to de a satisfactory test environment

edure

h subject had 5 trials of series of exposures ibration alone, and to vibration and noise ultaneously The subjects were given all the rimental conditions in the same order and the same trained physician Each trial was ormed from 6 00 to 9 00 p m at intervals weeks

efore exposure to noise or vibration, 4 min dy and interrupted cursive thresholds were ained from each subject After exposure, the iometric earphone was replaced, and the ject s threshold at 4 kHz was recorded as n as possible The post-exposure threshold asurements were conducted by the following er over 20 min duration

o reduce any fatiguing effects due to the tinuous test tone, the first tracings were for in, 40 sec (only the threshold tracings fol-ing exposure to noise alone were continu ly made for 4 min) and all of the subsequent

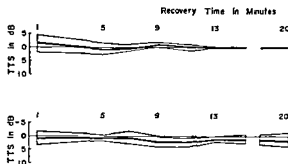


Fig 2

tracings were given with rests of 1 min alternating with tracings for 1 min The rest before the final tracing of 1 min was 5 min That is, the recovery process of the TTS was recorded 20 sec ($TTS_{0.3}$ or initial TTS), 3 min (TTS_3) 5 min (TTS_5), 7 min (TTS_7), 9 min (TTS_9), 11 min (TTS_{11}) 13 min (TTS_{13}), and 20 min (TTS_{20}) after stopping exposure

The cursive thresholds are represented by solid lines joining the midpoint of the excursion of the threshold tracings The identification of the resting threshold and these TTS values was made by averaging the first 10 sec periods in the solid line trace

RESULTS

For the magnitude and recovery of TTSs in each of the exposure conditions, the mean and the 95% confidence intervals of the TTS values obtained from all 7 subjects (except one with a pronounced auditory adaptation in the pre exposure threshold tone), were calculated and are shown diagrammatically in Figs 2 and 3 The resultant data are presented so that the open or stippled area has a width of one 95% confidence interval on either side of the mean

Effects of vibration alone

The upper section (I) in Fig 2 shows the results obtained following exposure to vibration of 300 cpm, and 6 mm amplitude for 20 min No shift in threshold sensitivity occurred in any of the measurements after exposure, but the first measurement after stopping the vibration showed

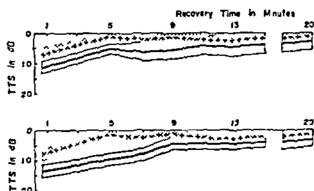


Fig 3

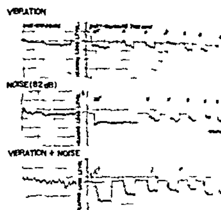


Fig 5

slight improvement in the threshold (about 3 dB lower than the resting threshold) However, the initial TTS showed considerable variation

The TTS values produced by exposure to vibration at a frequency of 1 000 cpm and an amplitude of 3 0 mm (the lower section (2) in Fig 2) showed only a slight change in threshold sensitivity at 4 kHz for 20 min, with a small increase of TTS_7 to TTS_{20} As shown in Fig 5, some of the subjects showed evidence of a loss of about 10 dB even in post-exposure tracings for 1 min

Comparison of effect of noise alone with effect of simultaneous noise and vibration

of the upper curves in both sections of 13 indicate the process of recovery from the elevated threshold following a 20 min exposure to a broad-band noise of 82 dB in SL The lower curves indicate recovery following exposure to

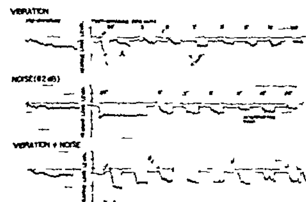


Fig 4

noise-plus-vibration of 300 cpm (upper (1)) or 1 000 cpm (lower section (2

The initial TTS produced by expos alone showed a mean threshold rise d and recovery to almost the pre-exposu old occurred in the short time of abou

Simultaneous exposure to vibration and noise caused the TTS to rise abo a significantly greater rise than af e to noise alone ((1) in Fig 3) Although illustrated in the figure that there w individual differences in the rate of r the pre-exposure threshold, the mea curve shows a much slower recovery th noise alone Actually the threshold normal 5 min after exposure, then rise again at 7 min

In many of the subjects considera TTS and delay of recovery was pro exposure to combined vibration at 10 and the 82 dB SL noise The mean T about 13 dB and TTS was 10 dB The r curves following noise alone and vibration were divergent for 9 min after stopped ((2) in Fig 3)

Thus, exposure to a noise of 82 dB to 1 000 cpm vibration simultaneously auditory sensitivity more than expo noise plus 300 cpm vibration or exp the noise alone

Although it is not shown in these f co-existence of noise and vibration cause a prolongation of tinnitus

little or no difference in the tone of the from that produced by exposure to lone Some of the subjects showed some e of the drop in short term tracings e cessation of exposure to vibration alone arly discernible change in the width of un tracings is not found when the attenuate is 1 dB/sec

4 and 5 show the changes of acoustic ity to the three exposure conditions of (No 7) over a period of 20 min

DISCUSSION

echanism of the injurious effect of vibra- the acoustic organ is very little under- From a review of the previous literature ; with the mechanical responses of the o low frequency vibration (Goldman & ke, 1961, Coermann, 1963, Miura, 1959), e attempted to elucidate the mechanism injury caused by forced vibration vibratory force of a frequency of 300 cpm produces motion of the head and dis- ient of the thoraco-abdominal organs ation does lower auditory sensitivity, it o so by both physical stimulation of the ic organs and physiological stimulation ng the thoracic and abdominal organs, can be considered responsible for a con- disturbance in homeostasis for autonomic nce

h a frequency of 1 000 cpm, the vibration ismitted from the excitation point to the o that the maximum resonance with in- ng accelerative force occurs in the head and The functional changes and the damage acoustic organ, probably including the l auditory nerve tissue, may be induced e physical factor of the vibratory force ng through the base of the skull and the jaw Because of the anatomical peculiarity inner ear, with its long, narrow, complex terioles, the disturbance following vibra- robably also affects the capillaries supply- ie auditory nerves, a classical example of ype of damage is Raynaud s phenomenon produced by local vibration in many workers using such equipment as pneumatic hammers and drills

In summary, it was suggested that the effects of the combined noise and vibration, if indeed they prove to be confirmed in future studies, might be the result of some complicated un- specified disturbances of physiological homeo- stasis or possible mechanical interactions with its blood supply—and let it go at that It is not made clear whether the present findings relate in any way to the animal experiments that are cited in the introduction

The results of these and of other experiments indicated that the simultaneous effect of vibra- tion-plus-noise is to increase the vulnerability of the auditory organ to the effect of noise alone, and the vibration alone has a negligible effect However, according to newer reports (Guignard & Coles, 1965, Jauhiainen et al , 1969), they indicated that whole body vibration had an enhancing effect on auditory function The effects of vibration on the auditory organ may vary with the experimental condition, it may be im- paired, unaffected or improved Many more studies are necessary before these effects can be elucidated in details

RÉSUME

Les changements de sensibilite auditive ont éte enregi- stres au moyen de la technique de l audiometrie de Bekesy en exposant a

1° les vibrations de 300 cpm (5 Hz) et 1 000 cpm (16 7 Hz)
2 le bruit blanc de 82 dB SL
3 les stimulations d ensemble de 1 et 2°

La vibration de frequence 300 cpm à 6 mm d ampli tude, ou la vibration de frequence 1 000 cpm à 3 mm d amplitude ont ete appliquees, jusqu à ce que le sujet n ait plus pu les supporter (pendant 20 min) par un excitateur à bruit et vibration contrôles On n a pas trouve de changements importants à la sensibilite du seuil apres n avoir expose qu aux vibrations En exposant aux vibrations et au bruit simultanement, les change- ments du seuil étaient plus grands et le temps de re- cuperation était plus long qu en exposant seulement au bruit C'etait particulierement remarquable pour la vibra- tion de 1 000 cpm On suppose que les effets combines du bruit et des vibrations pourraient être dus à la ten-

sion physiologique ou à la provision de sang De nombreuses autres études seront nécessaires pour en déterminer les effets

ZUSAMMENFASSUNG

Veränderungen der Hörfähigkeit wurden bei Schwingungen cpm bzw 1000 cpm oder Breitbandgeräusch bei 82 dB SL oder beidem mit einem Bekésy-Audiometer aufgezeichnet Mit einem Schwingungserreger mit Geräuscheinstellung wurde die Versuchsperson einer Schwingung, Frequenz 300 cpm, Schwingungsbreite 6 mm bzw 1000 cpm zu 3 mm ausgesetzt, solange sie es ertragen konnte, und zwar 20 Minuten

Bei Schwingungen allein waren keine signifikanten Veranderungen der Horgrenze zu verzeichnen Bei Schwingung und zugleich Gerausch entstanden grossere Veranderungen der Horgrenze (TTS), und die Normaliserung dauerte länger als bei Gerausch allein Das traf besonders bei einer Schwingung von 1000 cpm zu

Es ist zu vermuten, dass die Ergebnisse bei Gleich- [illegible]

Blutzirkulation sind

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-RETORY PROPERTIES OF CHRONICALLY INFLAMED MIDDLE EAR MUCOSA

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(Received November 22 1973)

-act Based on systematic examination of 13 chroni -infected temporal bones serially sectioned and -l for mucus the following conclusions concerning -al secretory properties were drawn (1) Chronically -ed middle ear mucosa showed increased secretory -ty in the form of goblet and other secretory cells -numbers of secreting cells were seen especially in -as of taller epithelium in places mucus positivity be demonstrated even in the ciliated cells In certain -the respiratory epithelium could even be replaced -cous epithel um Increased secretory function was be due partly to secretory metaplasia partly to -ry proliferation (2) Glands or gland like struc -were always present but in half the cases only their -rative forms were left The number and activity -glands depended upon the stage of the chronic -s When the situation had become chronic with -plastic mucosa the secretory elements increased the mucosa had become fibrotic (healing) even the *elements could degenerate and vanish In cases of* -plastic non healed chronic otitis the total number -nds in one ear seemed to amount to thousands a -undred being active (3) Clinically grossly diseased -ntory mucosa should be removed during chronic -rgery in order to avoid the development of secre -t tis and finally adhesive middle ears

-ite assiduous research, opinions on the -cteristics of normal middle ear mucosa are partly controversial Sade (1965, 1966*a*) light and Lim & Hussl (1969) using elec microscopy presented evidence that the -le ear lining is a true mucosa and has all -ecretory elements On the other hand -arzbart (1958) and Tos (1970) claimed that are no glands in the middle ear mucosa -fection of the tympanic cavity results in a -ict increase of secretory activity of the mucosa (Friedmann 1956, T Palva et al, 1964, Lim & Birck, 1971) In Sade s opinion the process was simple secretory hyperplasia while Hentzer (1972) favoured the idea of secretory metaplasia of tympanal epithelium Great numbers of secretory elements were seen in secretory as well as in chronic (suppurative) otitis media (Sade & Weinberg 1967, Hentzer, 1972) Bak Pedersen & Tos (1973) in their semi quantitative study, found the greatest density of glands in cases of secretory otitis media while in cases of chronic otitis the den sity was about 1/3–1/5 of that recorded in secretory otitis

The purpose of the present work was to study systematically in serial sections the secretory elements of middle ear mucosa in chronically infected temporal bones Using routine mucus stainings we tried to get a qualitative and semi quantitative picture of secretory elements and of their relation to tympanal pathology in gen eral in cases of chronic otitis media

-work has been financially supported by Emil -nen Foundation and by the Scientific Fund of -mentarium

MATERIAL AND TECHNIQUES

The material consisted of 13 chronically infected temporal bones with ear infections of from 4 months to 70 years duration The cadavers were kept in cold storage for 13–91 (mean 58 h) hours before the autopsy The temporal bones were fixed in 10 % formalin and decalcified with 22 5 % formic acid/sodium citrate solution After celloidin paraffin double embedding the blocks were sectioned in a frontovertical plane



Fig. 1 Tempor... Infero-medial w... anterior ... mucosa ... plasia. The ... stratified, ... plasia. S... are numerous ... structures and ... pherally a few ... cysts. One ... gland is also ... ×40

and the sectioning was started at the tubal orifice and continued posteriorly to the vertical part of the facial canal. Each 10 μ thick section stained for mucus, and with Haematoxylin-Eosin (H–E), represented sites 1.0 mm apart.

In addition to H–E staining for general characteristics, Periodic Acid Schiff (PAS) mucus staining was used, according to the MacManus method. We used this method both with and without diastase digestion. Diastase digestion time varied from 4 to 24 hours. The positive material stained magenta with PAS. Another mucus stain used was Alcian Blue (AB) without hyaluronidase phase. The positive material stained blue with AB.

A more detailed description of the ... and techniques is given in a pre... (Karma, 1973), based on the same ma...

In each section the characteristics of ... and epithelium were examined with l... scope using magnifications from ... Special attention was paid to AB- ... positivity of epithelium, to the ... secretory and goblet cells, to the ... and amount of glands, cysts and ... and to the activity of glandular ... thickness, oedema, and vascularity ... mucosa and the general characte... chronic process were noted.

The numbers of glands, cysts and ...



Fig 2 Temporal Bone 9 Infero-med al ep thelial lin ing of tubo tympanal area Two goblet cells are seen with a secretory columnar cell between them. The type of the ep thelium is pseudo-stratif ed ciliated and co-lumnar H E, 800 oil immersion

emi-quantified by counting the numbers i se structures per section in each individual oral bone

RESULTS

al characteristics (H–E)

ı previous work mentioned above, Karma ı) described the general epithelial charac cs of these ears In cases of chronic infec he middle ear epithelium showed in addi o an increase in its secretory capacity epi l hypertrophy (Figs 3 4) hyperplasia (Fig 1) and squamous metaplasia Immigrat ing squamous epithelium (Fig 16) epidermiza tion (Fig 16) and even epidermosis were en countered

As a rule respiratory, often secreting epithe lium lined the middle ear cavity widely in its anterior and inferior parts extending in some ears up to the posterior limits of the middle ear cleft The mucosa showed in all ears thickening oedema and vascularization of varying degree depending upon the inflammatory state of each ear Thickness of the mucosa varied from 0 05 to 2 0 mm but was usually about 0 5–1 0 mm

In addition to mucosal hyperplasia, there was in some cases marked organization, fibrosis and even tympanosclerosis

In 12 temporal bones the tympanal mucosa was infiltrated by inflammatory cells to a varying extent The predominant cells were lymphocytes and plasma cells but polymorphonuclear cells were also seen in many ears Varying numbers of macrophages were observed Temporal Bone 13 showed the most fibrotic mucosa in the whole material and the active inflammatory process had subsided. This was the only completely dry ear in the material

Secretory properties (PAS, AB)

The PAS staining with or without diastase digestion was similar in all sections and the length of the digestion time did not influence the staining Therefore, when speaking of PAS in the following, this refers to both staining methods

(*a*) *Glands* Real glands (Figs 1, 7–10, 19) were seen in 7 ears, the greatest number per section varying from 2 to 30 in each individual ear The number of glands was greatest anteroinferiorly and medially (especially in the window niches) diminishing postero-superiorly but they could be seen in all tympanal areas The size [illegible] the glands varied, being usually of the order [illegible] some tenths of a millimetre and they were [illegible]ually of simple tubular structure The proportion of active glands was at least 50% in five cases, in two it was clearly under 50% In three further temporal bones (1, 9, 11) only some degenerating glandular structures with dilated ducts and stagnant mucus were seen

(*b*) *Intra epithelial glands and epithelial pouches* A few real intra-epithelially located glandular initial stages (Fig 1) were seen in Temporal Bones 4, 11 and 12, gland-like pouches (Fig 11) lined by secretory epithelium appeared in 8 other ears

(*c*) Cysts (Figs 1, 8, 12–15, 19) occurred in all 13 ears and pseudocysts in 11, the maximum of each being up to 50 per section The infiltration of mucosa by cysts was most pronounced in Temporal Bones 4–11, while pseudocysts were more evenly divided [illegible] (see Table I)

(*d*) *Goblet cells* (Figs. 2, 3, 1[illegible] [illegible]gularly in 6 ears (Temporal B[illegible] [illegible]rregularly in four additional ea[illegible]

(*e*) *Mucoid epithelium* (Figs. 5–[illegible] [illegible]countered in 4 ears with greatest [illegible] in Temporal Bones 6 and 7 In 3 [illegible] this change was suggestive but not [illegible]

(*f*) *Secretory cells* (Figs 2–4, 1[illegible] [illegible]tion in the tympanum were seen [illegible] [illegible]cept one Both columnar and [illegible]tory cells appeared Ciliated cells als[illegible] showed mucus positivity (Fig 4). AB-positivity was most intense in the [illegible] (Figs 3, 4) of the epithelial cells.

(*g*) *Squamous epithelium* did not [illegible] secretory properties (Figs 14 17). In [illegible] of squamous epithelial metaplasia [illegible] AB positive response was demonstrated [illegible] superficially However, AB positivity [illegible] [illegible]ited to the area of slighter metaplasia [illegible]

Otherwise, the colour intensity [illegible] staining methods was usually equal [illegible] Temporal Bone 1 PAS positivity [illegible] [illegible]tinctly stronger

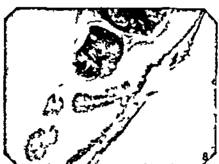
DISCUSSION

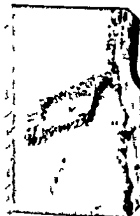
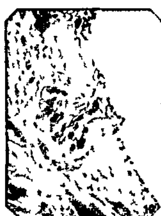
The constant lack of diastase effect [illegible] stainings means that there are no [illegible] in the mucosa of these sections [illegible] that polysaccharides (including [illegible] dissolved from the blocks in the [illegible]

Fig 3 Temporal Bone 3 Hypertrophic [illegible] thelium from hypotympanum. Goblet [illegible] secretory cells can be seen. Also some [illegible] show AB-positive material AB, 600 [illegible]
Fig 4 Temporal Bone 3 From the same [illegible] showing clearly the apically located [illegible] (secretory) material in the ciliated cells [illegible] respiratory epithelium. PAS 600 [illegible]
Fig 5 Temporal Bone 6 Mucous [illegible] lero-medial wall AB ×240
Fig 6 Temporal Bone 6 From the same [illegible] showing mucous epithelium. PAS 240
Fig 7 Temporal Bone 6 Active gland [illegible] wall of tympanum. PAS-positive [illegible] a small duct to tympanal lumen. [illegible] is mucously charged. PAS 94





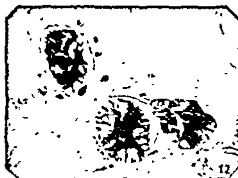


8 Temporal Bone 6 Infero-medial wall is lined by ous epithelium. Below a simple tubular gland is and above it two cysts The mucosal stroma has no positivity PAS ×96

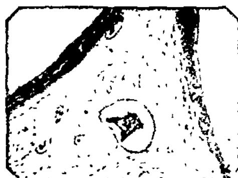
9 Temporal Bone 4 A simple gland from inferoial wall of posterior tympanum The stroma is rela y fibrotic and devoid of PAS positive material 150

Fig 10 Temporal Bone 7 A gland from medial tympanal wall in AB-staining AB 150

Fig 11 Temporal Bone 6 Infero-medial wall of anterior tympanum Mucous epithelium with gland like pouch formation Subepithelial stroma is relatively fibrotic H E, ×400











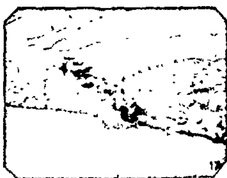






Fig 12. Temporal Bone 5 Cystic formations in the attic. AB, × 24

Fig 13. Temporal Bone 5 The same area as in Fig. 12, in PAS-staining × 24

Fig 14 Temporal Bone 12 Down from the inferior perforation edge. On the ear canal side, the epidermis does not stain with AB Cysts, tympanal epithelium and secretory blanket above it stain blue (AB-positive) AB, × 96

Fig 15. Temporal Bone 12 The same area as in Fig. 14 The PAS-staining is identical with AB PAS, × 96

Fig 16 Temporal Bone 8. In the upper [illegible] tympanum (near oval window area) the [illegible] epidermis changes to low columnar [illegible]. The epithelial junction is abrupt. The [illegible] dermis is AB-negative but ordinary [illegible] stains abundantly with AB. Medially there is [illegible] cyst having a faint bluish shade. AB, × [illegible]

Fig 17 Temporal Bone 12 The lower [illegible] The PAS-negative epidermis of the [illegible] abruptly at the border to tympanal PAS-[illegible]thelium PAS, × 240

tages Consequently, the PAS positive n is obtained only from the carbohydrate-complexes

generally equal coloration with AB and tains suggests that the secretion has a icous quality (Shackleford & Klapper, In those areas where PAS positivity was r than AB serous secretion with neu-ucosubstances apparently predominated

all degenerating and active glands as well ra-epithelial glandular structures were d, the total number of gland like forma-ose at the most to 110 per section in Tem-Bone 8 (see Table I) This means that the umber of glandular processes in the entire nal areas amounts to several thousands other hand, in Temporal Bone 13 there nly a few visible cysts as a sign of former ry activity In cases of secretory otitis Bak Pedersen (1973) counted the aver-mber of glands (all degenerative forms d) in the whole tympanal area and ar-at a figure of about 2 000, 93 % of the being active In their cases of chronic the mean density of the glands was 18-f the former and the percentage of active was only 8–20 of the total (Bak Pedersen , 1973) In the present material the num glandular structures and the proportion ve glands varied so greatly (see Table I) no meaningful average numbers can be The reason for this great variation is the at our material included ears in very dif-stages of chronic processes, from dry c to purulent oedematic mucosa How-the general impression was that in non-c ears the total number of all glandular ures was some thousands and active some hundreds per ear

greatest number of glands was usually n areas with marked mucosal hyperplasia (Figs 1, 19) Goblet and other secretory cells also appeared in larger numbers in the areas showing hyperplastic mucosa On the other hand, the ears with most active inflammation (Temporal Bones 1–3) and also showing pronounced mucosal oedema, had remarkably few secretory elements Ears with fibrotic mucosa (Temporal Bone 13) and ears with large areas of squamous epithelial lining (cf Karma, 1973) had only few secretory structures Thus in the acute stage (here acutization of a chronic process) the discharge is a result of both transudation and exudation The increase of secretory elements takes place predominantly when the inflammation has calmed down, resulting in both hyperplastic subepithelial stroma and epithelium

Cysts and pseudocysts were more numerous than the glands and they were more evenly distributed throughout the whole material This is explained by the long standing ear disease over the years, the active glands had degenerated, after acute exacerbations new glands had formed and again degenerated thus increasing the number of remarkably stable cystic structures In granulating areas many pseudocysts had formed while the flat tympanic epithelium attempted to cover the raw surfaces but lost the fight and was buried in the stroma by overgrowing granulation tissue After cessation of inflammation, cysts and pseudocysts may also lose their lumen and even the remaining epithelial strings may degenerate and vanish (see Temporal Bone 13)

Goblet and other secretory cells (see Table I) were usually seen in the areas with taller epithelium and in places the respiratory epithelium was wholly replaced by mucoid epithelium This means that there developed a change from the original function of transportation to secretion in the function of the epithelium Also ciliated cells showed mucous positivity in some areas especially in the apical parts of the cells (Fig 4) Both these findings suggest a mechanism of secretory metaplasia in cases of chronic otitis, in other words a metaplastic change of ciliated (and other epithelial) cells to secreting

Temporal Bone 7 Tympanal respiratory epi shows numerous goblet and columnar secretory B ×240

Temporal Bone 8 Oval window area There are lar and more peripherally cystic structures in erplastic mucosa PAS, ×40

Table I. *A synopsis of the findings*

Only positive findings are noted

The temporal bones have been numbered beginning from the ear showing most active inflammation and ending in the only dry ear (in which the active process is over) of the material. The identification of respective ears in the earlier work (*Karma, 1973*) *is given within parentheses. The proportion of active glands (a very rough proportion)* is to the so-called 'transitional varieties' (Tos & Bak-Pedersen, 1972), cysts and pseudocysts (Döderlein, 1920) are not counted to the proportion

Temporal Bone	1(3)	2(2)	3(1)	4(5)	5(4)	6(7)	7(8)	8(12)	9(13)	10(11)	11(10)	12(6)	13(9)
Age	70	56	85	59	59	70	51	83	58	83	51	11	70
Duration of symptoms	yrs	40 yrs	yrs	Minimum 4 months		?	From childhood		?	From childhood		1 yr.	?
Total absence of drum													
Total defect of tensa								+	+	+	+		
Subtotal defect of tensa							+						*
Central perforation	+			+	+	+						+	
Dry tympanum													+
Moist tympanum										+	+	+	
Purulent tympanum	+ + +	+ + +	+ +	+	+	+	+	+	+				
Thickness of *mucosa, mm*	0.5–1.0	0.5–1.0	0.5–1.0	0.5	0.5	0.1–1.0	0.1–1.0	0.1–1.0	0.1–1.0	0.3–2.0	0.1–1.5	0.1–1.3	0.05 0.2
Mucosal oedema	+ + +	+ + +	+ + +	+	+	±	+ +	+	+	+ + +	+ +	±	
Mucosal vascularization	+ +	+	+	+ + +	+ + +	+ +	+ +	+ +	+	+ +	+ +	±	
Secretion (AB & PAS +) in tympanum	+	±	+	+	+	+ +	+	+	+	+	+	+	
Inflammatory cell infiltration (degree of positivity is not evaluated)	+	+	+	+	+	+	+	+	+	+	+	+	
Follicular lymphocyte accumulations (degree of positivity is not evaluated)						+	+	+		+	+	+	
Cilia (number of strips)	+ (3)	±	+ (4)	+ (2)	+ (3)	+ (2–3)	+ (2)	+ (3)	+ (1)	+ (3)	+ (2)	+ (2)	+ (1)
Epithelial AB & PAS *positivity*	+ +	±	+	+ +	+	+ + +	+ +	+	+	+ +	+ +	+ +	
Secretory cells	+	±	+	+	+	+ + +	+ +	+	+	+ +	+ +	+	
Goblet cells	±	±	±	±	+	+ +	+	+	+	+	±	±	
Glands (ad number section)	+			+ (1–2)	+ (2–3)	+ (5–10)	+ (5)	+ (30)	±	+ + (10)	+ (5)	±	
Proportion of active glands	0			0	<1	<1	1	1	0	[illegible]	1	0	
Cysts (ad number section)	+ (5)	+ (5)	+ (5)	+ + + (50)	+ + (30)	+ + + (50)	+ + + (40)	+ + (50)	+ + (30)	+ + + (50)	+ + (20)	(5)	+
Pseudocysts (ad number section)	+ + (50)	+ (1–2)	+	+ + (20)	+ (5–10)	+ + (20)	(20)	+ (20)	+ (10)	+ (5–10)	+ (5)	+ + ([illegible])	
Intra-epithelial glands (p = ad [illegible] section, a = ad glands section)	(p5)	(p5)	(p1–3)	(p15) (a 2–3)	(p5) +	(p10)	(p10)	(p10)		(p5)	(p10) (a 7)	(p5) (a1)	

[illegible]

which mechanism was already proposed ɔoendlin (1959) and later confirmed by lectron microscopic findings of Lim & (1971) and Hentzer (1972) However, lering the very great number of secretory and glandular structures in some of our is compared with the smaller number of d cells in normals (Sade, 1966*a*), secretory eration must occur in addition to secre-netaplasia in cases of chronic otitis

se data on serial sections from chroni-inflamed middle ear mucosa have impor-clinical implications On the basis of the iour of squamous keratinizing epithelium, Palva et al, 1968, Karma, 1973) have em-ed the need for its complete removal from iddle ear We have felt, however, that in ase of non-keratinizing squamous epi-m, difficult to recognize without micro-examination, such thorough removal is quired since the possibly remaining small s of squamous metaplasia may revert to l middle ear mucosa so that no choleste-ı develops

present findings of thick mucosa, covered ucously changed epithelium and contain-ı some ears thousands of glands, cysts seudocysts in the stroma, suggest that this of mucosa cannot return to normal over and probably never does In other words e of this type of tympanum with a new l drum, even supposing that thorough ry of the mastoid and epitympanic space een made, is likely to lead to accumulation retions in the middle ear space analogous ive cases of secretory otitis media During ry, part of the middle ear mucosa is al traumatized with resultant raw areas In-n of fibrin in the thick seromucinous fluid the glands inevitably leads to the develop-ı of adhesive middle ears, or to non heal f the graft

ving witnessed the frequent development hesive middle ears we have for some years ised removal of grossly diseased thick nnar mucosa from the promontory, and ı covered the raw areas with Silastic sheeting Although we have not had a chance to study any of these ears later in serial sections, it may be presumed that a flat 1–2 cell layer of epithelium grows to cover the denuded areas under the Silastic Transportation of some secreted material, possibly formed in the hypotympanum or the tympanic roof, may occur by means of the cilia strips that may grow in the areas not covered with Silastic sheeting Similarly, thorough removal of suspected secretory epithelium from the posterior tympanum is mandatory as these secretions would not find their way out to the Eustachian tube in reconstructed and Silastic sheeting-covered middle ears

It has been suggested that the secretory activity of the middle ear mucosa after drum reconstruction may cease after insertion of grommets like those in the treatment of secretory otitis media In our experience this is not true and use of middle ear ventilation tubes is successful in the long run only if the major part of the pars tensa with its elastic layer is intact Also, mucosal changes in secretory otitis media are both by gross examination and in microscopic study considerably less pronounced than those of chronic long standing otitis media, and the time needed for the return to normal of the former may be several years Removal of the diseased mucosa is then on the way to healing

ZUSAMMENFASSUNG

An 13 chronisch entzundeten Schlafenbeinen wurden Serienschnitte gemacht und eine Schleimfarbung durchgefuhrt Aus ihrer systematischen Untersuchung konnten die folgenden Schlusse uber die sekretorischen Eigenschaften der Schleimhaut gezogen werden

1 Chronisch entzundete Mittelohrschleimhaut zeigte ein erhohtes Sekretionsvermogen in Form von Becherzellen und anderen sekretorischen Zellen Eine grosse Zahl sezernierender Zellen wurden speziell in den Bereichen des hoheren Epithels festgestellt stellenweise konnte ein positiver Schleimnachweis auch an Zilien tragenden Zellen gezeigt werden In bestimmten Bereichen konnte das respiratorische Epithel auch durch mukoses Epithel ersetzt sein Vermutlich beruhte die erhohte sekretorische Funktion teils auf sekretorischer Metaplasie und teils auf sekretorischer Proliferation

2 Drusen oder drüsenartige Strukturen waren immer vorhanden aber in der Hälfte der Fälle fanden sie sich

nur in degenerierten Formen Zahl und Aktivität der Drüsen hingen von dem Stadium des chronischen Prozesses ab Wenn die Situation chronisch geworden war, nahmen mit der hyperplastischen Schleimhaut die sekretorischen Elemente zu Wenn die Schleimhaut fibrotisch geworden war (abgeheilt war), kam es auch zu Degeneration und Schwund der zystischen Elemente In Fällen hyperplastischer, nichtverheilter chronischer Otitis schien die totale Zahl der Drusen in einem Ohr auf Tausende angewachsen zu sein, von denen ein paar Hundert aktiv waren

3 Klinisch gesehen sollte Promotoriumschleimhaut mit ausgeprägten Krankheitszeichen bei der Chirurgie der chronischen Mittelohrentzundung entfernt werden, um die Entwicklung einer sekretorischen Otitis und im weiteren von adhäsiven Mittelohrprozessen zu vermeiden

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FURTHER STUDIES ON ALTERNOBARIC VERTIGO

Posture and Passive Equilibration of Middle Ear Pressure

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r The passive equilibration of the middle ear ...e during simulated ascents was affected by different ...s and the level of the relative overpressure in the ... ear (P_{tm}) required to force the Eustachian tubes ...vas higher in the recumbent than in the sitting ...n Subjects with a high forcing pressure (FPL) in ...r and with experience of Alternobaric Vertigo ...xposed to simulated ascents in different postures ... P_{tm} recorded at the moment of onset of nystag... nystagmus pressure level NPL) was also higher in ...umbent position However an initially high FPL ...ot increased as regularly as an initially low FPL ... absence of vestibular stimulation in some subjects ...xamined in the recumbent position was explained fact that the FPL did not reach the NPL. A relation ...n the middle and the inner ear pressure being the ...uisite for vestibular stimulation is discussed

...well known that the pressure equalizing ...y of the Eustachian tubes is affected by ...ent positions of the body or the head ...e (1866) and Hartman (1878) studied this ...omenon by recording the pressure level ...red to open the tubes during Valsalva s ...euvre Perlman (1939) studied the influence ...ferent body positions on the patency of the ... of patients with tuba aperta, and found a ...ed patency in the recumbent position as ...pared with the erect position The reduced ...cy was assumed to be caused by lymphatic ...venous stasis in the vessels of the tubal ...osa The reduction of the pressure regulating ...city of the tubes in the recumbent position has later been confirmed by Rundcrantz (1969) in an investigation of subjects with eardrum perforation due to chronic otitis media or to trauma Ingelstedt et al (1967) could demonstrate the same phenomenon even in healthy military aircraft pilots

The assumption that venous stasis explains the reduced patency in the recumbent position was supported by a study of the pressure in the bulbus vena jugularis in different postures (Jonson & Rundcrantz, 1969) The pressure measured on the same level as the Eustachian tube was found to be about zero with the subjects seated and about 10 cm H_2O with the subjects in the recumbent position Ingelstedt et al (1967) also found that a pressure increase inside the internal jugular vein caused an increase of the volume of the middle ear mucosa as an expression of vascular filling of the mucosa A corresponding volume increase of the tubal mucosa might consequently be a possible explanation of the reduced patency of the Eustachian tubes in the recumbent position or during neck vein compression

It has been demonstrated that vertigo might be elicited in otologically healthy subjects by a relative overpressure of a certain level, induced in the middle ear by lowering of the ambient pressure (Ingelstedt et al, 1974) The subjects were instructed to avoid active clearing of the middle ear pressure during the ambient pressure decrease, and the magnitude of the induced overpressure was dependent upon the level of

...investigation was supported by grants from the ...

the passive forcing pressure, i e the pressure required to force the Eustachian tubes open passively In the same work asymmetry was also demonstrated between the forcing pressure level on the right and the left hand side The importance of the magnitude of this asymmetry for the vestibular stimulation could, however, not be evaluated

The aim of the first part of the present work was to study the influence of different body positions on the passive equilibration of the middle ear pressure at simulated ascents

METHOD AND EQUIPMENT

The following symbols are used in text and figures

P_{atm} atmospheric pressure on ground
P_m pressure in middle ear
P_{tm} pressure gradient across eardrum
P_{ch} pressure in chamber, i.e ambient pressure
V_m volume of airfilled middle ear space
V_{tm} volume displacement of eardrum in relation to its neutral position
$\dot{V}_{tm}$ airflow through resistor of the ear canal flowmeter caused by volume displacement of eardrum
$\dot{V}_{ec}$ airflow through resistor of the ear canal flowmeter, caused by expansion and compression of gas volume in external ear canal and in flowmeter system by changing the ambient pressure
$\dot{V}_{ref}$ airflow through resistor of the reference flow meter, caused by expansion and compression of gas volume in reference system by changing the ambient pressure
Δ before the symbol indicates a change of the variable
Pressure is expressed in cmH_2O volume in microlitres (μl) or millilitres (ml) and airflow in microlitre sec (μl/sec)
P_m and P_{ch} are relative to atmospheric pressure on the ground

Fig 1 gives an outline of the equipment used for the recordings A pressure chamber was used in which the subjects were exposed to simulated ascents of 90 cmH_2O during which they were instructed to avoid active equilibration of the middle ear pressure The ambient pressure decrease caused a relative overpressure in the middle ear and there was no pressure equilibration until the relative overpressure was great enough to force the Eustachian tubes open passively (forcing pressure level FPL) A method (Ingelstedt et al , 1967, Elner et al, 1... which it is possible to record the volume... ment of the eardrum (V_{tm}) caused by the overpressure in the middle ear (Fig... method made it possible to determ... sure change in the chamber (ΔP_{ch}) re... force the tubes open passively A pol... catheter with a rubber disc was inserted... inner bony part of the external ear c... tightly connecting the space between the... and the disc with the measuring de... method is based on recording the airflo... by the eardrum displacement (V_{tm}) by... a flowmeter The airflow through t... meter consists both of the flow $\dot{V}_{tm}$... and $\dot{V}_{ec}$ has to be eliminated for an... recording of $\dot{V}_{tm}$ This elimination is p... using an identical flowmeter with an a... reference volume $\dot{V}_{tm}$ is then integrated... is recorded

The relative overpressure in the mid... the moment just before the tube is for... is, however, not equal to the pressure... the chamber (ΔP_{ch}) at the same mome... has been shown that there is only a sm... ence between P_{tm} and ΔP_{ch} in the case o... cally healthy subjects, i e subjects with... or a large middle ear volume (>6 ml, ... et al , 1974)

In the first part of the present study th... pressure was given as ΔP_{ch} For a co... mination of the pressure gradient ac... eardrum (P_{tm}) at the moment of passive... it is necessary to know ΔP_m as well ... induced during the simulated ascent and ... at the moment of passive forcing Th... be calculated as ΔP_{ch}–ΔP_m For a... description of the method and the calcul... ΔP_m, see Ingelstedt et al (1967 and 1... Elner et al (1971*a*)

Eye movements were recorded simul... with the pressure recordings by means of... nystagmography (ENG) and the record... performed in total darkness with the ... eyes open (Tjernstrom 1973) The sub... also instructed to report any feeling of ...

·I *Forcing pressure level (FPL) of the right and the left ear, recorded during simulated ascents* …assive *clearing of the ears in the sitting, the recumbent and the left and the right lateral supine* …ons

[1] PL is given as ΔP_{ch}, i.e. the pressure change in the chamber required to induce a relative overpressure in the … ears high enough to force the tubes open passively

		Right ear			Left ear		
		FPL (ΔP_{ch}, cmH_2O)			FPL (ΔP_{ch}, cmH_2O)		
…t	Position	Mean	Range	No of exams	Mean	Range	No of exams
[illegible]	Seated	31.3	28.5–34.5	9	18.4	18.0–19.5	9
	Recumbent	46.0	43.5–48.0	10	35.0	33.0–37.5	10
	Right lateral	50.3	48.0–52.5	2	36.0	35.5–36.5	2
	Left lateral	48.0	45.0–51.0	3	40.5	36.0–43.5	3
[illegible]	Seated	37.7	32.0–43.0	11	49.0	41.0–58.0	11
	Recumbent	51.5	49.0–55.0	6	60.2	57.0–65.0	5
	Right lateral	57.0	56.0–58.0	3	60.0	58.0–62.0	3
	Left lateral	51.3	49.0–53.0	3	64.7	63.0–66.3	3
[illegible]	Seated	35.7	34.0–38.0	11	31.3	26.0–35.0	11
	Recumbent	57.5	53.0–60.0	6	51.5	46.0–54.0	6
	Right lateral	65.5	64.5–66.5	2	48.5	46.0–51.0	2
	Left lateral	58.5	58.0–59.0	2	62.5	61.0–64.0	2
[illegible]	Seated	41.3	38.0–45.0	9	33.2	31.2–36.0	9
	Recumbent	53.0	52.0–54.0	4	44.0	42.0–46.0	4
[illegible]	Seated	42.8	39.0–46.0	11	38.9	34.0–46.0	11
	Recumbent	51.6	50.0–53.0	5	45.2	43.0–46.0	5

…e flow-volume measuring unit and the …ure measuring unit were calibrated before …after every examination according to the …nal method Eye-movements were cali…d for 30° in each direction before and after …/ test

…rimental procedure

…subjects were exposed to simulated ascents …passive clearing of the ears The examina… …were performed with 5 subjects in the …d and the recumbent position Furthermore, …the subjects were also examined in the left …right lateral supine positions The subjects …med each position for about 5–10 minutes …re the examination started

MATERIAL

…material consisted of 5 healthy subjects in …age range 22–34 years, 2 of whom women and …n All subjects were regarded as otologically normal with no history of ear disease and a normal ear examination The hearing threshold was within 0–20 dB (related to ISO standard, 1964) All subjects were without signs of catarrhal infection at nose and throat examination The subjects had a normal active tubal function (groups I*b* and I*c*, according to Elner et al., 1971*b*) All of them were also known to have a forcing pressure of a normal level (30–50 cmH_2O, see Ingelstedt et al , 1974) They all had a middle ear volume of both ears >6 ml

RESULTS

In Table I the FPL of right and left ears are given, recorded with the subjects in different postures The FPL was found to be higher in the recumbent than in the sitting position The mean increase was about 13 cmH_2O (mean of all means) The FPL was also affected by the different lateral positions and the recordings showed a higher FPL in the ear which was turned down-

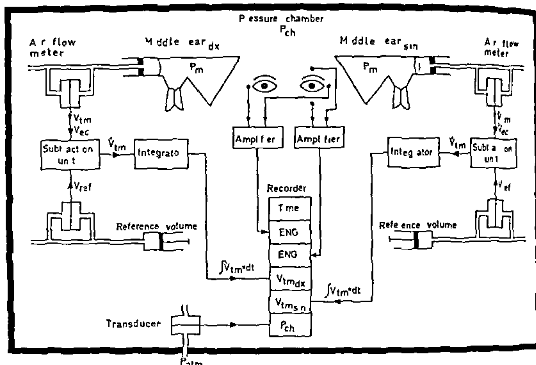


Fig 1 Outline of the equipment used for recordings of eardrum displacement (V_{tm}) at changing ambient pressure (P_{ch}) The equipment as well as the subjects are placed in the pressure chamber For more deta ls see text symbols

wards than in the ear turned upwards No sign of vestibular stimulation was observed

COMMENT

The results presented in Table I which show that a higher FPL is required to force the tubes open in the recumbent position, are in agreement with earlier investigations into the patency of tubes in different positions (Lucae, 1866, Hartman, 1878, Perlman 1939, Ingelstedt et al, 1967, Rundcrantz 1969) However, there seems to be no published report of the magnitude of the FPL increase due to changes of posture It is also seen from the results that it might be possible to vary the FPL asymmetry between the ear by examinations in different lateral positions

It has been demonstrated (Ingelstedt et al, 1974) that certain subjects have a particularly high FPL in one ear and a lower FPL in the other These pressure conditions were proved not to be chance findings as it was possible to reproduce them at repeated exam na intervals of several months 5 subjects with a high FPL (>60 cmH_2O) reported when exposed to simulated ascents and clearing of the ears in the sitting posit these 5 subjects reported vertigo during ascent and 3 only occasionally appeared every time vertigo was reported nystagmus was also observed in several although the subjects did not recogn bular stimulation One subject never vertigo and no nystagmus was observed though this subject also had a high FPL

The aim of the second part of the work was to study

(*a*) the possibility of increasing forcing pressure by change of body pos to find out if a higher relative overpress the middle ear causes a stronger ves sponse,

(*b*) if a subject with a high forcing level but without feelings of vertigo in th position gets vertigo and/or nystagmus

II *Forcing pressure level (FPL) of the right and the left ear, recorded during simulated ts and passive clearing of the ears in the sitting and the recumbent positions*

Position	Right ear FPL (P_{tm}, cmH_2O) Mean	Range	No of exams	Left ear FPL (P_{tm}, cmH_2O) Mean	Range	No of exams	Vertigo incidents/ No of ex	Nystagmus incidents/ No of ex	Velocity of slow nystagmus phase (°/sec)
Seated	58 4	54 6–62 4	3	37 6	33 9–40 7	3	3/3	3/3	0 5–1 0
Recumbent		67 9	1		59 6	1	1/1	1/1	2 0
Recumbent + neck compression	73 7	71 8–75 3	3	—	—	—	3/3	3/3	1 0–2 0
Seated	63 0	61 3–65 6	5	50 9	46 0–56 0	5	0 5	0 5	—
Recumbent	72 4	69 9–74 5	7	62 0	60 3–66 1	6	1/7	5/7	1 5–3 0
Seated	70 6	65 4–74 1	6	47 6	43 1–50 2	6	1/6	1/6	0 5
Recumbent	72.6	67 3–79 3	4	56 2	53 0–58 7	4	0 4	0 4	—
Seated	70 4	68 1–73 4	9	47 6	38 6–54 4	9	1/9	3/9	0 5–1.5
Recumbent	74 5	71 8–76 5	3	54 4	46 9–66 5	3	0 3	0 3	—
Seated	63 7	62 5–65 4	5	34 2	33 2–46 7	7	3/7	3/7	1 0–1 5
Recumbent	63 1	61 1–66 9	3	52 0	49 6–55 3	3	0,3	0/3	—
Seated	60 8	59 4–62.2	3	66 1	65 2–67 9	3	1/3	2/3*	2 0–3.5
Recumbent	58 6	57 8–59 4	2	60 1	55.2–65 0	2	0 2	0/2	—

mbent position when the forcing pressure is higher,

) the possibility of inducing a variation in the ing pressure asymmetry between the left and right ear in order to evaluate the importance ie asymmetry in the mechanism of Alterno-c Vertigo

ethod, equipment and experimental procedure

method is described above and the equip-t is presented in Fig 1. The FPL was given P_{tm} and the calculations, which require knowl- of the middle ear volumes (V_m), were per-ned as described by Ingelstedt et al (1974) subjects had a middle ear volume >6 ml, rmined indirectly by converting the area of airfilled middle ear system, measured from ay films, to a volume (Andreasson, 1973) subjects were exposed to simulated ascents he sitting and the recumbent positions. One ject was also examined in the left- and the it-hand lateral position, and in two experi-nts (see Results) the neck veins were compressed by means of an inflatable cuff (Jonson & Rundcrantz, 1969) The cuff was inflated to 40 cmH_2O by a pressure fan

Material The material consisted of 6 healthy subjects aged between 22 and 26 years, all of whom were men The subjects were known to have a high FPL on one side (>60 cmH_2O) in the sitting position, and a forcing pressure asymmetry between the ears which differed from one subject to another All subjects were regarded as otologically normal with no history of ear disease and a normal ear examination The hearing threshold was within 0–20 dB (related to ISO standard, 1964) The subjects were without signs of catarrhal infection at nose and throat examination and there was no history of labyrinthine disease The subjects had a normal active tubal function (groups I*b* and I*c*, according to Elner et al, 1971*b*) All subjects but one (subject VII) had reported vertigo when exposed to simulated ascents and passive clearing of the ears in the seated position Subject VII had had

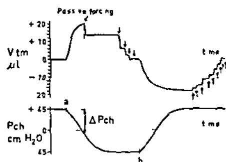


Fig 2 Recording of eardrum displacement (V_{tm} in µl)

ear pressure is equilibrated with the chamber pressure ($V_{tm}=0$) Then the examination starts (*a*) with a simulated ascent (pressure decrease of 90 cmH_2O) The Eustachian tube is forced open passively (passive forcing) the relative overpressure in the middle ear is then reduced (the eardrum moves inwards) and the middle ear pressure is equilibrated to the chamber pressure by deglutitions (*arrows*) Before the start of another examination the chamber pressure is increased (*b*) and the eardrum moves inwards due to a relative underpressure in the middle ear The underpressure is equilibrated with the chamber pressure by deglutitions (*arrows*) and it is checked that the eardrum is in its neutral position before start of the next examination

no feeling of vertigo despite a high forcing pressure on one side

Results The relative overpressure in the middle ear (P_{tm}) was calculated at two different moments during the ascents, i e at the moment when the Eustachian tube was forced open passively (FPL) and at the moment when the onset of nystagmus was recorded (NPL = nystagmus pressure level) In Table II the FPL on the right and the left hand side is given, in the sitting and the recumbent positions, together with the number of vertigo incidents and the intensity of the vestibular response (velocity of the slow nystagmus phase)

In subjects VI and VII a bilaterally higher FPL in the recumbent position was recorded and the increase was of about the same magnitude as in the subjects presented in Table I The recordings of subjects VIII and IX showed a bilaterally higher mean FPL in the recumbent position However, some overlapping isolated results were also seen The rise in FPL was less pronounced in the ear with a high FPL … ear) than in the ear with a low FPL (… In subject X the mean FPL was found about the same in the right ear (… irrespective of posture while an FPL … was recorded on the left hand side (low … the recumbent position Subject XI had … FPL on both sides when examined in the … position, but the posture change did n… any increase in FPL On the contrary th… ings showed a lower value of the FPL … recumbent position

In all subjects but one (subject VII) … as well as vertigo appeared when the … were examined in the sitting position S… reported vertigo in both positions (sit… recumbent) and the vestibular response … even to be a little stronger in the supine … No sign of vestibular stimulation appear… subject VII was examined in the sitting … but in the recumbent position vert… reported once and nystagmus appeared

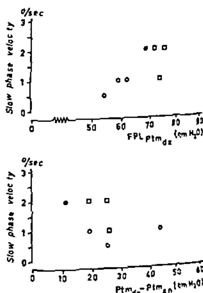
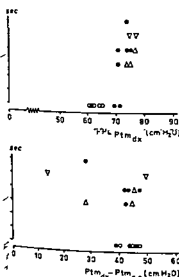


Fig 3 Relation between the intensity of the … response (max velocity of the slow nys… and the forcing pressure level (FPL) (upper p… *part* Relation between the intensity of the … response and the pressure asymmetry betwee… and the left ear ($P_{tm_{dx}} - P_{tm_{sin}}$) at the mome… … open The f… … were … • = occur… … tion ☐ = recumbent position and … n occ…

examinations Subjects VIII, IX, X and XI ...ted no vertigo when examined in the recum... position and no nystagmus appeared

...bject VI was also examined in the recumbent ...ion during neck vein compression in order ...luce an additional FPL increase, which was observed, and both nystagmus and vertigo ...ared Subject VII reported vertigo when ...ined in the left- and right-hand lateral posi... and nystagmus was also recorded The ...s of the examinations of subjects VI and ... all performed at one session, are presented ...gs 3 and 4 The upper half of the figures ...s the relation between the vestibular response ...agmus) and the FPL The lower halves ... the relation between the pressure asym... (difference between the relative over...ure in the right and the left middle ear, ...$-P_{tm_{sin}}$, at the moment when the right ...achian tube is forced open) and the vesti... response

...though the findings are not statistically con...ve the graphical presentation in Figs 3 and ...ms to indicate a stronger vestibular response ...igher FPL It is also indicated that the ...itude of the pressure asymmetry between



...4 The figure shows examinations of subject VII all ...hich were performed at one session, and the same ...ons are presented as in Fig. 3 O = seated position, ...ecumbent position, Δ = right lateral supine posi... ∇ = left lateral supine position

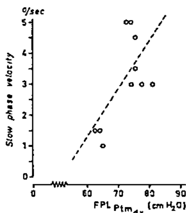


Fig 5 Relation between the intensity of the vestibular response and the forcing pressure level (FPL) The figure shows the examinations of subject X, performed in the seated position at different sessions The line of short dashes represents a calculated regression line

the ears did not seem to affect the intensity of the vestibular stimulation when the asymmetry exceeded about 10 cmH_2O

It seemed surprising that no sign of vestibular stimulation was recorded when subjects VIII, IX and X were examined in the recumbent position, in spite of the fact that in this position the recorded FPL was apparently high enough to stimulate the vestibular system in the sitting position In view of these results, the pressure level required to cause a vestibular stimulation in the recumbent position seemed to be higher than that in the sitting position In order to test this assumption the NPL recorded in the sitting and the recumbent positions of subject VI was calculated This calculation showed that the mean NPL was about 8 cm higher in the recumbent position than the mean NPL in the sitting position, and the NPL was even higher when the examinations were performed in the recumbent position during neck vein compression In order to confirm the results presented above, repeated examinations of subjects VI, VII, VIII and X were performed in the sitting and the recumbent position, and subject VII was also examined during neck vein compression in the recumbent position

Table III shows the results of the re-examinations and the FPL is given for the ear with the

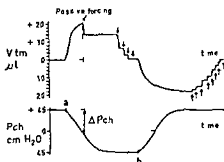


Fig 2 Recording of eardrum displacement (V_{tm} in μl) and chamber pressure (P_{ch} in cmH_2O) Eardrum movement outwards (−) and inwards (−) Each examination starts with a simulated descent with a pressure increase of +45 cmH_2O after which it is checked that the middle ear pressure is equilibrated with the chamber pressure (V_{tm} 0) Then the examination starts (*a*) with a simulated ascent (pressure decrease of 90 cmH_2O) The Eustachian tube is forced open passively (passive forcing) the relative overpressure in the middle ear is then reduced (the eardrum moves inwards) and the middle ear pressure is equilibrated to the chamber pressure by deglutitions (*arrows*) Before the start of another examination the chamber pressure is increased (*b*) and the eardrum moves inwards due to a relative underpressure in the middle ear The underpressure is equilibrated with the chamber pressure by deglutitions (*arrows*) and it is checked that the eardrum is in its neutral position before start of the next examination

no feeling of vertigo despite a high forcing pressure on one side

Results The relative overpressure in the middle ear (P_{tm}) was calculated at two different moments g the ascents, i e at the moment when the istachian tube was forced open passively (FPL) and at the moment when the onset of nystagmus was recorded (NPL nystagmus pressure level) In Table II the FPL on the right and the left hand side is given, in the sitting and the recumbent positions together with the number of vertigo incidents and the intensity of the vestibular response (velocity of the slow nystagmus phase)

In subjects VI and VII a bilaterally higher FPL in the recumbent position was recorded and the increase was of about the same magnitude as in the subjects presented in Table I The recordings of subjects VIII and IX showed a bilaterally higher mean FPL in the recumbent position However, some overlapping isolated results were also seen The rise in FPL was less pronounced in the ear with a high FPL ear) than in the ear with a low FPL In subject X the mean FPL was fo about the same in the right ear irrespective of posture, while an FPL was recorded on the left hand side (low the recumbent position Subject XI ha FPL on both sides when examined position, but the posture change did any increase in FPL On the contrary th ings showed a lower value of the FPL recumbent position

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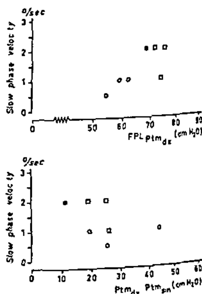


Fig 3 Relation between the intensity of the response (max. velocity of the slow nystag and the forcing pressure level (FPL) (*upper p part*) Relation between the intensity of the response and the pressure asymmetry betwe and the left ear ($P_{tm_{dx}} - P_{tm_{sin}}$) at the mome right Eustachian tube is forced open. The fi examinations of subject VI all of which were at one session O = sitting position ● = recum tion □ = recumbent position and neck version

-equired to cause a vestibular stimulation in the recumbent position, as the tubes forced open passively by a lower pressure ıan the NPL However, as is seen from the III the FPL of subject X was much higher one of the sessions, and in this case mus as well as vertigo appeared in the bent position, too The same was also ed in subject VIII in one of the re-ex- tions

e 5 gives a graphical presentation of all nations of subject X performed at different ıs in the sitting position The figure shows lation between the FPL and the intensity *vestibular response (velocity of the slow* mus phase)

DISCUSSION

ressure level required to force the Eustach be open passively was affected by different ons of the body in agreement with the ral effects on the tubal function (Ingelstedt 1967, Rundcrantz, 1969) It seems pro- that the postural effects on the FPL is of vascular filling of the tubal mucosa due reased hydrostatic venous pressure in the nbent position (Jonson & Rundcrantz, Rundcrantz, 1969) This assumption is er confirmed by the experiments with neck compression, which caused an additional increase It seems reasonable to suppose the postural effects on the tubal mucosa is ar to that on the middle ear mucosa (Ingel et al, 1967, *Rundcrantz, 1969) However,* hange of posture did not affect an initially FPL as regularly as an initially low FPL, in one subject a slight FPL decrease was observed in the recumbent position

ne level of the relative overpressure in the lle ear (P_{tm}), recorded at the moment of t of nystagmus (NPL), was, however, also d to be higher in the recumbent than in the ed position An additional NPL increase furthermore observed when the examinations performed during compression of the neck veins The NPL increase seemed to be even more regular than the FPL increase

The intracranial pressure is known to increase by about 10–13 cmH_2O when the posture is changed from the erect to the horizontal position (Best & Taylor, 1945) If the cochlear and/or the endolymphatic ducts have a pressure-equalizing function (Allen, 1964, House, 1964, Holden & Schuknecht, 1968), the intralabyrinthine pressure would be expected to rise when the intracranial pressure is increased Since the increase of the intracranial pressure is mainly an effect of a hydrostatic venous pressure increase (Dawson, 1967), it might be assumed that the intralabyrin- *thine pressure would also rise as a result of the* same mechanism even without any pressure- equilibrating function on the part of the ducts

In a previous study (Tjernström, 1974) it was found possible to elicit approximately the same vestibular response by overpressure in the middle ear regardless of whether the overpres- sure was induced locally in the middle ear via a transmyringeal tube or indirectly by means of an ambient pressure decrease (intact eardrum) The prerequisite for vestibular stimulation seemed to be a certain relation between the middle ear pressure and the ambient pressure The results of the present study, however, indi cate that in actual fact the prerequisite might be a certain relation between the inner ear and the middle ear pressure This assumption is based on the observation that the NPL was always higher in the recumbent than in the sitting posi tion and in view of the different mechanisms discussed above in connection with the intra- *cranial and the intralabyrinthine pressure, the* NPL increase in the recumbent position might be due to an inner ear pressure increase This assumption is also confirmed by the observation that a still higher NPL was recorded at the examinations in the recumbent position during neck vein compression However, there is also an increase in arterial pressure in the recumbent position which must be taken into account when discussing the mechanism of A V

The way in which overpressure in the middle ear affects the vestibular system is still unknown

Displacement of the inner ear fluid and disturbance of the blood circulation are possible factors A circulatory disturbance could be due to an increased intralabyrinthine pressure affecting the arterial as well as the venous blood flow The overpressure in the middle ear might also affect blood vessels which communicate with the inner ear and thus disturb the normal blood flow

It is known that impulses are regularly emitted by the resting vestibular organ, and a decrease in the discharge in one ear causes a nystagmus directed towards the other, while an increase in the discharge directs the appearing nystagmus towards the affected ear It has been demonstrated (Ingelstedt et al, 1974) that the nystagmus observed in the experiments of A V is always directed towards the ear with the highest FPL Thus it seems that the overpressure in the middle ear somehow causes an increased discharge of impulses from the affected ear Such an activation might reflect a genuine stimulation following for example an utriculopetal cupula deviation in the horizontal semicircular canal or be due to a disturbance of the neurophysiological mechanism responsible for the discharge of resting vestibular impulses (pseudo-stimulation)

The results of the present study have indicated a tendency in the vestibular stimulation to increase in intensity with higher FPL values (cf Figs [illegible] and 4) However, the FPL increase as demonstrated in the figures was induced by a change in posture, and it could not be evaluated to what extent the posture change *per se* might have contributed to the indicated nystagmus increase From the recordings of subject X, however, different forcing pressure levels were observed in the sitting position at different sessions and as seen from Fig 5 the nystagmus intensity was higher in the experiments when a higher FPL was recorded It could not be assessed, however, whether the indicated stronger stimulation was due to a higher P_{tm} or to a prolonged stimulation time in the examinations where the passive opening of the tube occurred at a later moment during the ascent

In the present study, pressure asymmetry between the right and the left ear [illegible] observed, but asymmetry *per se* did [illegible] vestibular stimulation unless the [illegible] pressure in one ear reached a [illegible] (NPL) Furthermore, the magnitud[illegible] asymmetry did not seem to affect [illegible] of the vestibular stimulation The [illegible] not statistically conclusive, however [illegible] studies have to be performed

ZUSAMMENFASSUNG

Der passive Druckausgleich im M[illegible] simulierter Steigungen wurde durch die Kör[illegible] beeinflusst In liegender Stellung war ein hoher[illegible] Überdruckniveau im Mittelohr (P_{tm}) als im S[illegible] wendig um die Eustachischen Tuben zwang[illegible] offnen

Personen mit hohem (zwangsmässigem) Öff[illegible] (FPL) in einem Ohr, die früher alternobare[illegible] erfahren hatten, wurden simulierten Steig[illegible] setzt, und zwar in verschiedenen Stellungen [illegible]

[illegible] so regelmässig wie ein geringer, und das [illegible] vestibularer Stimulation bei einigen Persone[illegible] der Untersuchung im Liegen konnte [illegible] werden, dass der FPL nicht das Niveau des NPL [illegible] Ein Zusammenhang zwischen dem Druck im M[illegible] und im Innenohr als Voraussetzung fur die [illegible] Stimulation wird diskutiert

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ADRENERGIC INNERVATION OF THE NASAL MUCOSA IN CAT

A Histological and Physiological Study

A Änggård and O Densert

From the Department of Pharmacology Karolinska Institutet the Department of Otolaryngology, Karolinska sjukhuset and the King Gustaf V Research Institute Stockholm Sweden

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Abstract The adrenergic innervation of the cat nasal mucosa was investigated with fluorescence and electron microscopy A strong fluorescence attributable to adrenergic nerves was observed on the outer surface of the smooth muscle layer of blood vessels whereas the fenestrated capillaries showed no evidence of adrenergic innervation A sparse adrenergic innervation was observed in connection with the mucosal glands The effects of sympathetic nerve activation and various denervation procedures were evaluated with a local tracer disappearance technique reflecting the vascular events in the exchange vessels of the maxilloturbinal area Both functional as well as morphological data pointed to an ipsilateral distribution of the sympathetic nerve fibres from the superior cervical ganglion to the nasal mucosa The sympathetic postganglionic nerve fibres to the nose travel via the tympanic plexus After passage through the tympanic plexus the major sympathetic nerve supply to maxilloturbinal area travels in the Vidian nerve

The functional consequences of autonomic nerve activation have been intensively studied in cat nasal mucosa (Malcomson, 1959, Malm, 1973*a*, *b*, Änggård & Edwall 1974, Änggård, 1974) However, the majority of the morphological investigations of the autonomic innervation of the nasal mucosa have been performed in human material (Dahlstrom & Fuxe, 1965, Cauna, 1970, Cauna et al, 1972, Ishii, 1970, Ishii & Toriyama, 1972) Thus the interrelationship between morphological findings and functional effects of autonomic nerve activation of the nasal vascular bed has not been the subject of a systematic study in one species

This investigation has been supported by the Swedish Medical Research Council (Grant no 40X 3518)

From previous morphological stud[illegible] been assumed that the adrenergic [illegible] restricted to blood vessels and that th[illegible] lack an adrenergic innervation (Dah[illegible] Fuxe, 1965, Cauna et al, 1972) This la[illegible] adrenergic innervation of the glands [illegible] nasal mucosa is at variance with what is [illegible] known from other glandular tissues (E[illegible] 1968), and could possibly be due to species [illegible] tion or differences in the techniques [illegible] autonomic innervation of the nasal glands [illegible] therefore be reexamined in the cat [illegible] improved technique (cf Hokfelt, 1968 [illegible] & Thoenen, 1967)

In patients suffering from "vasomotor [illegible] a Vidian neurectomy has been perform[illegible] reduce the parasympathetic activity to th[illegible] mucosa, thought to be responsible for symptoms of increased nasal secre[illegible] congestion in this condition (cf Änggård [illegible] From experimental studies in dogs a[illegible] there is still no agreement as to whethe[illegible] Vidian nerve contains sympathetic as [illegible] parasympathetic fibres to the nose (Tsch[illegible] 1913, Blier, 1930, Malcomson 1959 Ja[illegible] Rooker, 1971, Malm, 1973*a*) Expe[illegible] evidence suggesting a strong sympath[illegible] ence on the nasal vascular bed has been [illegible] from functional studies in the cat (Äng[illegible] Edwall, 1974) If at Vidian neurectomy [illegible] thetic as well as parasympathetic fibres [illegible]

-energic denervation of the nasal mucosa result, with a decrease in the sympathetic constrictor tone

he present study, the adrenergic innervation of the cat nasal mucosa has been examined light and electron microscopy to provide for physiological studies in this species onal and morphological results have been ited after sympathetic denervation, by -ation of the superior cervical ganglion, -mpanic plexus, or the Vidian nerve

MATERIAL AND METHODS

iments were conducted on 11 cats (1 5 to g) In 2 cats only morphological studies performed

sectioning

enervations were performed under general iesia (Nembutal i p 30 mg/kg) one to three before the experiments

ilateral sympathectomy was performed in by extirpation of the superior cervical ion The tympanic plexus was cut via the d tympanic bulla on one side in 2 cats and oth sides in 2 cats The Vidian nerve was ed through a transorbital approach and cted on one side in 3 cats

r disappearance measurements

cats were anesthetized with chloralose-ane (50 mg/kg+100 mg/kg i v) and the ea was cannulated The pressure in the ral artery was measured using a Statham ure transducer (P23A) and recorded on a multichannel recorder Rectal temure was kept constant at 38°C by heating s The technique used to measure changes e microcirculation is the same as described previous paper (Änggård & Edwall, 1974) ssence, vascular events in the exchange ls are studied by measuring the local disarance rate of an easily diffusible tracer, from a local depot in the maxilloturbinal of the nasal mucosa Radioactivity was counted for periods of 40 sec. The disappearance rate (*k* value), which represents the fractional elimination of the depot per minute, was calculated on a computer for each time interval When sympathetic stimulation was performed the cervical sympathetic nerve was dissected free from the vagal nerve and transected The nerve was stimulated in the cranial direction using a bipolar silver electrode The stimuli were monophasic square wave pulses (6 V, 1 msec) delivered by a Grass model S4 stimulator Stimulation frequencies were varied in random order

Fluorescence microscopy

After the functional studies the anesthetized animal was decapitated and pieces of the nasal mucosa were taken from the septum, the maxilloturbinal, ethmoturbinal and nasoturbinal area The specimens were freeze-dried and treated with formaldehyde gas for the demonstration of biogenic amines according to the histochemical method of Falck & Hillarp (for ref see Corrodi & Jonsson, 1967) A Zeiss fluorescence microscope with a dark field condenser was used Photomicrographs were taken using Scopex G film

Electron microscopy

In order to facilitate the demonstration of sympathetic adrenergic nerve terminals by electron microscopy, two cats were pretreated with 5-hydroxydopamine (3,4,5-trihydroxyphenylethylaminehydrochloride) 4×20 mg/kg i p over a period of 48 hours (Tranzer & Thoenen, 1967) Before decapitation the animals were anesthetized with Nembutal (30 mg/kg i p)

Thin slices from the nasal mucosa were fixed in ice-cold 3% potassium permanganate in 0 1 M sodium phosphate buffer at pH 7 0 for 45 minutes (Richardson, 1966, Hokfelt, 1968) After rinsing in Ringer solution, the specimens were dehydrated in ethanol and embedded in Epon (Luft, 1961) Ultrathin sections were cut with a diamond knife on an LKB ultratome The sections were briefly contrast-stained in lead citrate (Reynolds, 1963) Electron microscopy was performed using a Siemens Elmiskop I

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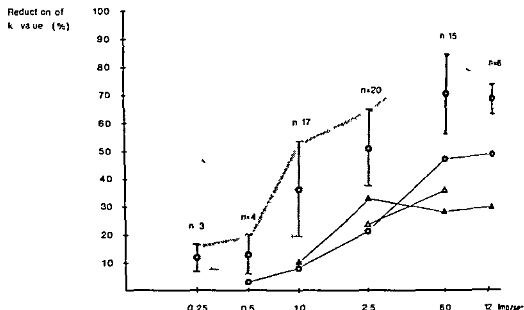


Fig 1 The influence of sympathetic nerve stimulation on tracer disappearance rate after Vidian neurectomy (3 cats) The shaded curve represents the normal responses found in a previous study of non-denerva cats) (Änggård & Edwall 1974)

RESULTS

Tracer disappearance measurements

Upon stimulation of the ipsilateral sympathetic nerve, the tracer disappearance rate on the non-denervated side of the cat was reduced to the same degree (Fig 1) as was found in a previous ˌ (Änggård & Edwall, 1974) After a ateral chronic sympathectomy no reduction ... acer disappearance rate was seen on stimula ion of the contralateral sympathetic nerve In 3 out of 6 cats in which the tympanic plexus had been cut a complete transection was achieved In these animals no response was recorded following nerve stimulation

After transection of the Vidian nerve in 3 cats, stimulation of the ipsilateral cervical sympathetic nerve resulted in reduced responses which were in all cases significantly smaller than the normal sympathetic responses (Fig 1)

Fluorescence microscopy

In all parts of the normal nasal mucosa investigated there was a rich fluorescence typical of sympathetic adrenergic nerves Blood vessels of various sizes were surrounded by a large number of adrenergic nerves (Fig 2A, B) Fl was particularly prominent arour t and venous sinusoids In some ca varicosities could not be distinguishe of the heavy innervation Below the e small blood vessels, evidently arteri seen surrounded by an adrenergic ne In this region some adrenergic ne seemed to end freely just below the membrane The nerve fibres did not the membrane and no fluorescence was inside the epithelium In the glandula the mucosa a sparse network of f fibres was seen to surround the sepa (Fig 2A)

Unilateral cervical sympathectomy a complete ipsilateral disappearan fluorescent fibres (Fig 3) while fluorescence remained on the contrala When the tympanic plexus was cut a cence on the operated side disappear proximal end of the cut tympanic intense accumulation of fluorescent lamines was observed Some fluores observed even at the distal end In the tympanic plexus was found to be inc





The adrenergic innervation in the nasal mucosa laxilloturbinal area Fluorescence is most apparent d arteries and sinusoids A sparse network of fluorescent nerve fibres is observed around the glands (↗) (B) Ethmoturbinal area Fluorescence not as prominent as in the maxilloturbinal area

ted In such cases a normal fluorescence seen, as well as a normal response on tracer ppearance rate on sympathetic stimulation all cats with Vidian nerve transection a ced and scattered fluorescence was observed ie maxilloturbinal mucosa The remaining escence was most apparent around the r arteries (Fig 4) In the nasoturbinal and ioturbinal area, fluorescence was unaffected

tron microscopy

energic nerve terminals were easily recog d with the technique employed Apart from chondria, the terminals contained a varying iber of dense cored vesicles with a diameter bout 500 Å and a few large granulated vesicles a diameter of about 1 000 Å Nerve termi- containing exclusively small non granulated cles were seen They had the appearance of cholinergic terminals, as described by Grillo (1966) and will be referred to as cholinergic

In the mucosa mostly unmyelinated bundles of nerve fibres were seen These unmyelinated axons were located in groups and enfolded in a Schwann cell In these bundles it was difficult to distinguish between adrenergic and cholinergic nerve fibres, since many fibres contained no vesicles The extensive adrenergic innervation of the blood vessels was revealed by the presence of terminals containing granular vesicles Rows of adrenergic nerve terminals were located on the outside of the smooth muscle layer around the arteries, arterioles, and sinusoids (Fig 5) The terminals were partly enfolded in a Schwann cell, with their free surfaces facing the smooth muscle fibres Scattered cholinergic terminals containing agranular vesicles were regularly observed together with adrenergic terminals

They were enfolded by the same Schwann cell (Fig 6) Fenestrated capillaries were present under the respiratory epithelium and around the mucosal glands Neither cholinergic nor adrenergic innervation could be demonstrated around these vessels

The principal innervation of the blood vessels was adrenergic in nature, whereas the majority of nerve terminals in the innervation of the nasal glands contained small agranular vesicles, considered to be cholinergic Bundles of nerve fibres and terminals were seen among the nasal glands and in interacinar spaces The axons were partly or completely surrounded by a Schwann cell Most terminals contained agranular vesicles, but in almost every group of terminals there were one or two containing dense cored vesicles considered to be adrenergic in nature (Fig 7) In the glandular parts the cholinergic nerve terminals outnumbered the adrenergic terminals by roughly 10 to 1, while the reverse r found around the blood vessels



Fig 3 Nasal septum after an ipsilateral cervical sympathectomy No fluorescence observed

DISCUSSION

In the present investigation techniques the function as well as the morphol adrenergic innervation of the cat nasal were used

The present tracer disappearance has previously been used to study effects of sympathetic and para nerve activity in the cat nasal mucosa nique reflects the vascular events in turbinal area of the nasal mucosa (cf Edwall 1974, Änggård 1974) The lished histochemical fluorescence Falck & Hillarp (for ref see Corrodi & J 1967) provides a possibility of visual



Fig. 5. Electron microscopic picture of an artery in the nasal mucosa. Adrenergic nerve terminals (*A*) located along the smooth muscle fibres (*M*). Lumen (*L*). Cholinergic terminal (*arrow*). ×6 400.



Fig. 6 Higher magnification of framed area in *Fig 5*, illustrating adrenergic (*A*) and cholinergic (*C*) nerve terminals along a smooth muscle fibre (*M*) ×22 750

…ic neurons and thus demonstrating the …ects of sympathetic denervation The speci…ity of the technique and the fact that the fluorescence completely disappeared following superior cervical ganglionectomy suggested that the fluorescence in the nasal mucosa represents postganglionic sympathetic nerve fibres

Postganglionic transection results in a degeneration of the distal nerve axons and resulted in the abolition of fluorescence Transsection of the tympanic plexus thus resulted in a complete ipsilateral sympathectomy and accumulation of transmitter in the proximal end of the cut nerve, indicating that the postganglionic sympathetic nerve fibres constitute a part of the tympanic plexus in the cat This is at variance with what is assumed to be the case in man, where the postganglionic fibres are considered to form an internal carotid plexus and then, as the deep petrosal nerve, join the parasympathetic fibres in the Vidian nerve to continue furth… nasal mucosa (Ritter, 1970) Howev… anatomical difference might be expla… embryonic regression of the internal c… artery as occurs in the cat, where the… the adult cat is replaced by a fibrou… (Walker, 1972)

The conflicting reports on the symp… nerve content of the Vidian nerve (B… Malcomson, 1959; Jackson & Rook… Malm, 1973*a*) were suggested by Jack… Rooker to be due to a spread of the st… current to surrounding tissues on Vi… stimulation With the present histoch… technique it could be clearly demonstr… the maxilloturbinal area in the cat is symp… cally innervated via the Vidian nerve, wh… nasoturbinal and ethmoturbinal areas are… vated along other pathways After a… neurectomy the fluorescence was spars…



7 Electron microscopic picture of the glandular [ ] of the nasal mucosa Nerve fascicle between glandular cells (*Gc*) contains adrenergic (*A*) and cholinergic (*C*) nerve terminals ×32 500

[ ]illoturbinal vascular bed and sympathetic [ ]ulation resulted in only slight reductions in [ ]er disappearance rate This indicates that [ ] remaining sympathetic innervation affected [ ] exchange vessels in the maxilloturbinal area, [ ]ough to a lesser degree

[ ] a rhinomanometric study of the cat, Malm [ ]73*a*) found an increase in nasal patency when [ ]ulating the contralateral sympathetic nerve [ ]s is at variance with the results of the present study where no fluorescent fibres were seen to cross the midline and no effect was seen on the exchange vessels on contralateral sympathetic stimulation However, if a collateral circulation does exist, a leakage of the adrenergic transmitter to the blood stream (cf Fuxe & Sedvall, 1965) might explain this variance in results As a relative difference exists in the degree of the vasoconstrictor response between the exchange and capacitance vessels in the nasal mucosa



Fig 6 Higher magnification of framed area in Fig 5, illustrating adrenergic (*A*) and cholinergic (*C*) nerve terminals along a smooth muscle fibre (*M*) ×22 750

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(Änggård & Edwall, 1974), a vasoconstrictor response would most likely appear in rhinomanometric measurements, which mainly reflect the capacitance function of the nasal mucosa

Previous morphological studies on the nasal mucosa in man and various experimental animals have failed to demonstrate an adrenergic innervation of the glands (Cauna et al, 1972, Dahlström & Fuxe, 1965) In the present study on the cat, however, it was observed that, apart from blood vessel innervation, there is also a sparse adrenergic innervation around the acini in the mucosal glands This variance in results can probably be ascribed to the use of different techniques for the demonstration of biogenic amines The present ultrastructural technique used permanganate as a fixative, as suggested by Richardson (1966) and further developed by Hokfelt (1968) Furthermore, pretreatment of the cat with the 'false" transmitter 5-hydroxydopamine was used to increase the levels of amines in the adrenergic storage vesicles (Tranzer & Thoenen, 1967), thus facilitating the identification of adrenergic neurons Thus almost all large and small vesicles in adrenergic terminals contained a dense core In the glandular parts the majority of nerve fibres appeared empty or contained agranular vesicles, described y Grillo (1966) as cholinergic However, nerve ascicles around and in between the acini contained nerve terminals with dense-cored vesicles as evidence for an adrenergic innervation as well

The autonomic dual innervation of the nasal glands corresponds to what is known from salivary glands (cf Emmelin, 1968, Norberg et al, 1969) This similarity between salivary glands and the nasal glands might indicate that the physiological aspects of autonomic nerve activation might be similar as well, though the sparse adrenergic innervation suggests that cholinergic effects may dominate the secretory responses within the nasal mucosa

ACKNOWLEDGEMENTS

Skilful technical assistance was given by Mrs Sonja Bjornberg and Mrs Marie Louise Spångberg

ZUSAMMENFASSUNG

Die adrenerge Innervation der Nasenschl[illegible] Katze wurde fluoreszenz und elektronen[illegible] untersucht Eine kräftige Fluoreszenz, [illegible] durch adrenerge Nerven wurde an der [illegible] flache der glatten Muskelschicht der B[illegible] tet wahrend die gefensterten Kapillaren ke[illegible] [illegible]

verschiedener Denervationsmassnahmen [illegible] Hilfe einer lokalen Tracer-Abkling Tech[illegible] welche die vaskulären Auswirkungen in den [illegible] fässen der Maxilloturbinalregion widersp[illegible] funktionelle als auch morphologische Erge[illegible] auf eine ipsilaterale Verteilung der sympathis[illegible] fasern vom Ganglion cervicale sup zur Nasensc[illegible] Die sympathischen postganglionären Nerve[illegible] laufen uber den Plexus tympanicus zur [illegible] Durchtritt durch den Plexus tympanicus [illegible] Hauptanteil der sympathischen Nerven[illegible] Maxilloturbinalregion auf dem Wege des [illegible] Nerven

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β-ADRENERGIC RECEPTORS IN THE VESSELS OF THE CAT NASAL MUCOSA

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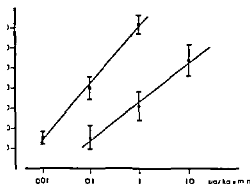
Abstract The effects of two close arterially given β receptor stimulating agents isoprenaline and terbutaline on resistance and capacitance vessels in the nasal mucosa of the cat were investigated in terms of measurements of mucosal venous blood flow and changes in nasal patency, respectively Both drugs decreased blood flow resistance (perfusion pressure / venous blood flow) and nasal patency, i e dilated resistance and capacitance vessels In equi weight doses isoprenaline was more effective than terbutaline in decreasing the blood flow resistance and the nasal patency The β receptor blocking agent propranolol reduced the effect of isoprenaline and terbutaline on the resistance vessels and caused a parallel shift of the semi logarithmic dose–response curves of both drugs It is concluded that the effects of isoprenaline and terbutaline in the nasal vascular bed of the cat are mediated via β adrenergic receptors at least in the resistance vessels

& Jackson (1968) studied the behavior of nasal vessels in terms of changes in nasal ...cy in the dog in response to β receptor stimulating and β receptor blocking agents They concluded that the nasal vessels of the dog lack β adrenergic receptors In an investigation on cats (Malm, 1973*a*), in which both the nasal venous blood flow and the nasal patency were studied, the dilator effects of isoprenaline seemed to be reduced by the β receptor blocking agent propranolol Although the number of observations in that study was small, the results suggested the existence of β receptors in the cat nose vessels The present investigation is a more thorough study of this problem, in which the effects of two β receptor stimulating a... prenaline and terbutaline, were stud... and after propranolol administration.

The present study was supported by grants from C O Lundberg s Foundation and the Swedish Medical Research Council (B74-14X 2210-08C)

METHOD

The observations were made on cats, ... anaesthetized with α-chloralose (about ... i v) after induction with ether The anim... tracheotomized Venous blood flow and ch... in nasal patency were recorded from the ... nasal cavity using methods which h... described previously (Malm, 1973*b* 19... brief, the vein leaving the nasal mucosa ... pterygopalatine foramen was cann... blood flow recording For this purpose ... venous blood flow from the nasal ... diverted through an optical blood flow ... unit and then returned to the animal via a ... connected to the right jugular vein The ... flow was continuously recorded on ... paper The height of the venous outfl... ter was set to 6 to 8 cm above the p... tine foramen to obtain about normal ... outflow pressure (Malm, 1974) Chan... nasal patency were estimated in terms of ... changes in a thin walled water-filled ... placed in the right nasal cavity The ba... connected via a polyethylene tubing ... tronic pressure transducer (EMT 3... Schönander) for recording on a polygr... gograph M81, Elema-Schönander) In th... trol state the pressure in the balloon ...



Mean changes (+S E M) of nasal blood flow ice after i a infusions of different doses of isopre (×) and terbutaline (●) in 7 cats The ordinates nt the decrease of blood flow resistance as a per of control resistance in a linear scale and the doses of the drugs in a logarithmic scale Re n lines are calculated from the mean changes by st squares method ($p<0.01$ for both correlation ents)

of H_2O Arterial blood pressure was re- d from the right femoral artery via a mer- nanometer on the kymograph Close arte- fusions were given through the right lingual in retrograde direction via a polyethylene er and with the aid of a constant infusion Venous injections were given in the right al vein Just before the insertion of the ters the cats were heparinized The cervical athetic nerve was acutely cut on the right efore the observations were made

gional vascular resistance was calculated by ng the perfusion pressure by the venous l flow The responses of the resistance ls in the nasal mucosa to the drugs were ssed as the per cent change of vascular ance from the control value prevailing e drug administration Pressure changes in alloon have been shown to be linearily d to changes of nasal patency and can be as a semi quantitative measure of evoked nses in the capacitance vessels in the nasal sa (Malm, 1974) The capacitance responses expressed in terms of pressure changes in alloon (cm H_2O)

e following drugs were given isoprenaline tate, terbutaline sulphate, propranolol hydro- de and papaverine sulphate

Paired comparisons and Student's t test were used in the statistical analyses

RESULT

Isoprenaline and terbutaline were given in a series of 7 cats close arterially to the nasal mucosa as infusions of as a rule 1 min duration Four doses of each drug were generally administered Isoprenaline 0 001, 0 01, 0 1 and 1 μg/kg b w × min and terbutaline 0 01, 0 1, 1 and 10 μg kg b w × min The lowest dose of the two drugs was nearly always subthreshold In suprathreshold doses the two drugs always evoked an increased venous flow and a decreased nasal patency and in response to the highest dose of each drug often a detectable decrease of arterial blood pressure The responses of the resistance vessels were expressed as the percentage decrease of blood flow resistance below the control level and the results are summarized in Fig 1, which gives mean values ± S E M The difference between the vascular resistance changes evoked by equi weight doses of isoprenaline and terbutaline was significant ($p<0.001$) Regression analyses (the least squares method) showed that the dose–response curves were linear ($p<0.01$ for both correlation coefficients) in semi logarithmic plotting From these two regression lines the dosages of the two drugs required to evoke the same blood flow resistance change may be deduced The relative effectiveness of the two drugs on a weight basis can thereby be estimated For example, to evoke a 20 % decrease of vascular resistance, the dose had to be 13 times larger for terbutaline than isoprenaline

The effects of these two drugs on the nasal capacitance vessels were also studied with the balloon technique The balloon pressure always increased, indicating a dilatation of the capacitance vessels, after suprathreshold doses of the two drugs The effects of both drugs were relatively small On a equi-weight basis, however, isoprenaline was more effective than terbutaline, the difference being significant, but only at the $p<0.05$ level The dose–response curves were

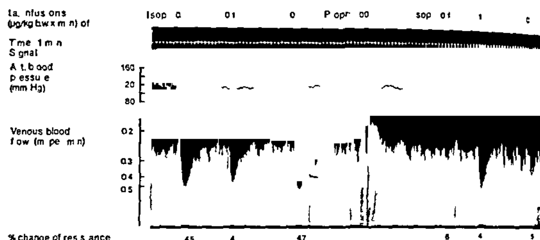


Fig 2 Cat 2 5 kg Effects of ısoprenal ne (Isopr) ı a before and after propranolol (Propr) ı a on arterıal blood pressure and nasal venous blood flow Infus are marked below the t me record.

not lınear ın semı logarıthmıc plottıng ($p>0\ 05$ for the correlatıon coeffıcıents) why no data on the relatıve effectıveness of the two drugs ın changıng nasal balloon pressure can be gıven

These results evoked by two β receptor stımulatıng agents suggest the presence of β adrenergıc receptors ın nasal resıstance and capacıtance vessels The problem was therefore further ınvestıgated with the aıd of the β receptor blockıng agent propranolol admınıstered close arterıally (4 cats) to the nasal mucosa as well as ıntravenously (6 cats)

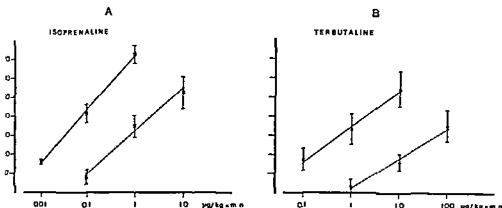
The magnıtude of the venous blood flow ın ase evoked by ısoprenalıne and terbutalıne was found to be reduced by propranolol and an example of thıs can be seen ın Fıg 2 In thıs cat three repetıtıve ınfusıons of ısoprenalıne (0 1 μg/kg b w × mın ıntra arterıally) elıcıted quıte sımılar ıncreases of venous blood flow (dılatıons of the resıstance vessels) before propranolol admınıstratıon After propranolol (100 μg/kg b w × mın for 3 mın ıntra arterıally) ısoprenalıne ın the same dose as mentıoned evoked a much smaller ıncrease of blood flow An about tenfold ıncrease of the dose of ısoprenalıne was now requıred to produce as large dılator effects of the resıstance vessels as before propranolol The calculated decreases of the flow resıstance are also gıven ın Fıg 2 below the tracıngs

The bulk of data ın thıs serıes of experıments was obtaıned after ıntravenous admınıstratıon of propranolol ın a dose of 1 3 mg/kg pronounced arterıal blood pressure fall ensued upon the ıntravenous ınject on of propranolol Isoprenalıne and terbutal ne were ever not gıven unt l the blood pres returned to near normal values The results these experıments are summarızed ın F (mean values ± S E M) Panel A refers prenalıne and panel B to terbutal ne the solıd lınes passıng through the mean effects ın the resıstance vessels before dashed lınes after propranolol ad It can be seen that propranolol caused parallel shıft of the dose–response curve rıght The dıfference for equı weıght dose sıgnıfıcant at the $p<0\ 001$ level for ısopr and at the $p<0\ 02$ level for terbutalıne

The capacıtance responses (balloon p ıncreases) evoked by terbutal ne were ly reduced by propranolol ($p<0\ 05$) those evoked by ısoprenal ne were not s cantly affected ($p>0\ 05$)

These studıes wıth the β receptor b substance propranolol further support the that β adrenergıc receptors ex st ın the bed of the nasal mucosa at least ın the res vessels

The possıbılıty remaıns that propran addıtıon to ıts β receptor blockıng ef a non specıfıc actıon on the resıstance In an attempt to elucıdate thıs problem



Mean changes (±S E M) of nasal blood flow re ... e in per cent of control after i a infusions of differ... ses of isoprenaline (panel A) and terbutaline (panel ... ore (——) and after (----) propranolol 1–3 mg/kg i v in 6 cats Ordinates and abscissa are as in Fig 1 Regression lines are calculated from the mean changes by the least squares method ($p < 0\ 02$ for all four correlation coefficients)

ive study (three cats) was performed with ıer dilator substance, papaverine, which has receptor stimulating effect Isoprenaline and verine were infused in doses which evoked oarable dilatations of the resistance vessels ie control state After propranolol admin-ition papaverine was about equally effective lating the resistance vessels in contrast to effect of isoprenaline which was clearly ced

DISCUSSION

present investigation shows that the two ceptor stimulating substances, isoprenaline terbutaline, dilate the resistance vessels in the l mucosa of the cat The magnitudes of these ations were clearly reduced by propranolol iermore, propranolol seemed to cause a llel shift to the right of the lines, which in logarithmic plotting represented the dose–onse curves of isoprenaline and terbutaline, ctively These parallel shifts may suggest a petitive antagonism The results thus speak ivour of the existence of β-adrenergic recep in the resistance vessels, this concept was ier supported by control experiments with dilator agent papaverine

ie two β receptor stimulating substances al-lilated the capacitance vessels Propranolol ied to reduce the magnitudes of the capacitance responses to the two drugs, but in this material the reduction was statistically significant only for terbutaline It thus appears that β adrenergic receptors are present also in the nasal capacitance vessels, although perhaps less abundantly than in the resistance vessels Such an uneven distribution of the β receptors has previously been discussed for the vascular bed of skeletal muscle (Mellander, 1960, Johnsson & Öberg, 1968)

Hall & Jackson (1968) could not selectively block the responses to isoprenaline on the nasal patency in dogs with propranolol and therefore they suggested a lack of β-adrenergic receptors in the nasal vessels With their method, however, the effects were only observed in the capacitance vessels and their conclusion thus has to be restricted to events in this type of vessel in the dog The present results clearly indicate the presence of β-adrenergic receptors in the nasal vessels but, as mentioned, perhaps mainly in the resistance vessels Of course it cannot be excluded that species differences exist between the dog and the cat

Lands et al (1967) have classified β adrenergic receptors in different groups designated β_1 and β_2 According to their concept, heart muscle and intestinal smooth muscle contain β_1 receptors and smooth muscle of the respiratory tract, blood vessels, uterus and diaphragm β_2-recep-

tors Isoprenaline stimulates both β_1 and β_2-receptors, terbutaline affects mainly β_2-receptors (Persson & Olsson, 1970) These latter authors found that isoprenaline in equi-weight doses was as much as 200 times more effective than terbutaline in increasing the contractile force by 20% in heart muscle of the cat (β_1-receptor effect), whereas it was only 20 times more effective in reducing peripheral blood flow resistance in the cat hind limb or lower half of the body (β_2-receptor effect) Since isoprenaline in this study was 13 times more effective than terbutaline in reducing nasal blood flow resistance by 20%, it appears that these β-receptors belong to the β_2-type It should be stressed, however, that the classification in β_1- and β_2-receptors has been criticized in recent years Thus, Brittain et al (1970) consider it not necessary to postulate a diversity of β receptors as they do not vary greatly in their sensitivity to the natural catecholamines noradrenaline and adrenaline

Isoprenaline and terbutaline are drugs used in man, and especially terbutaline has a beneficial effect in bronchial asthma The question whether therapeutical doses of the two drugs significantly could affect the human nasal blood flow and patency may be raised in view of the present esults and should be elucidated by future investigations

ACKNOWLEDGEMENTS

Generous supply of terbutaline sulphate from Draco AB, Lund, Sweden, is gratefully acknowledged

ZUSAMMENFASSUNG

Der Effekt von zwei β Rezeptor stimulierenden Substanzen, Isoprenalin und Terbutalin auf die Resistanz und Kapazitanzblutgefässe in der Nasenschleimhaut von Katzen wurde durch Messung der venösen Durchblutung in der Nasenschleimhaut und Veranderungen im Nasenraum untersucht Beide Substanzen, in der Nahe der Nase arteriell infundiert, verminderten den Widerstand der Durchblutung und verursachten eine Ansch... Nasenschleimhaut, die Substanzen ... Resistanz und Kapazitanzblutgefasse Isopr... sich als effektiver, wenn beide Substanzen ... Mengen gegeben wurden Die β blockierende ... Propranolol reduzierte den Effekt von Isopr...

Terbutalin im nasalen vaskularen Bett der ... mindest in den Resistanzblutgefassen, via β-... Rezeptoren vermittelt wird

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MUCOCILIARY WAVE PATTERN

An analysis of surface light reflections

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act Experiments were performed regarding surface reflections obtained from the rabbit trachea in vitro a standardized method using a microscope photo-pl er technique Comparative experiments have been on a water surface with induced surface waves result of these studies revealed that the surface tions obtained from mucous membranes of the atory tract are composed of at least two com-nts i e from the mucous surface and from the t of cilia beneath the surface

ciliary activity of the respiratory epithelium be examined indirectly via variations of reflections recorded from the illuminated ous membrane surface (Mercke et al, 1974) irectly with the use of intracellular electrodes kansson & Toremalm, 1966) Intracellular rdings are valuable from a theoretical point iew, but cannot be used for longterm experi-ts Indirect methods are therefore best d for applied experimental research The are intimately associated with the medium which they work, therefore, the chemical physical properties of the mucous layer are important The light reflections may arise a the mucous layer and from the surface lia beneath the surface The ciliary activity lied via surface light reflections should be e correctly described as *mucociliary wave uency* rather than as *ciliary beat frequency*, term currently utilized

investigation has been supported by grants from the Ish Medical Research Council, Project number 14X 3897-01

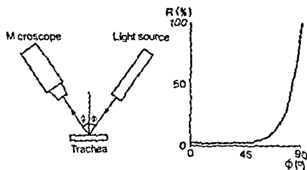
The object of the present research has been to investigate and compare surface reflections from mucous waves induced by mucociliary activity of the rabbit trachea in in vitro experiments and surface reflections initiated by a vibrating tone probe on a water surface in order to analyse the origin of reflections The following questions are considered

1 Do the recorded light reflections appear in the mucous layer alone?
2 Do the light reflections appear from the surface of cilia?
3 Do the reflections have a double origin?

METHODS

Recording of mucociliary waves

The mucociliary activity on the surface of rabbit trachea has been recorded by an indirect light reflection method for in vitro experiments as described in detail in a previous paper (Mercke et al, 1974) A light beam was aimed at the inner surface of a rabbit trachea mounted in an experimental chamber Variations in light surface reflection brought about by movements of the cilia in the covering mucous layer were focused in a binocular microscope The optical signals were converted to electrical pulses by a photo-multiplier attached to one of the ocular tubes The pulses were magnified and interfering frequencies e g from the mains were eliminated by



The relat onsh p

a clipping filter The light reflection variations were instantaneously recorded

Recordings were also made from the trachea through a 12 μ thick transparent polythene plastic film and a 150 μ thick piece of glass, respectively The mucous layer was covered in this way in order to distinguish between reflections from the true mucous waves of the secretion layer and reflections obtained from the waves of sweeping cilia just below the surface

In all experiments the temperature and relative humidity parameters were kept constant (38°C, r h 90–100 %)

cording of artificial waves

A tone probe connected to a tone generator (Hewlett Packard 202 C) was submerged in ordinary tap water in a plexiglass case Waves with a frequency of 10, 15 or 20 Hz were induced on the water surface The wave patterns were picked up with the method described above The tone generator output was recorded simultaneously on an oscilloscope screen f… parison

The waves induced on the water su… also in this case investigated when a 12 … transparent polythene plastic film or a … thick piece of glass was kept floating by … tension forces in front of the mic… Recordings of light reflections we… through each of these covering material…

Theoretical calculations regarding surface reflections and wave movements

The experimental design is illustrated … in Fig 1 Varying intensities of reflect… are obtained according to correspond… tions of the angle between the incident … the reflected light

The relationship between the intensity … reflected light (R) and the angle of … (ϕ) is illustrated in the diagram show… right in Fig 1 It is presumed that the … of the secretional waves are small as co… with the wave length (Barnett & … Therefore the angle of incidence var… little From this it is clear that the light … variations also are reduced due to the … slight slope of the curve $R(\phi)$ when ϕ < 4…

Since the light is directed against a … secretions, the reflection takes place on a … surface Therefore, the reflected light … either *converged* or *diverged* depending … configuration of the surface A concave … convex reflecting surface is illustrated in F…

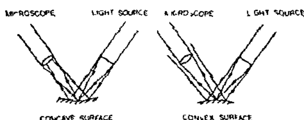


Fig 2 Light reflections from a wave trough (*left*) and a wave ridge (*right*)

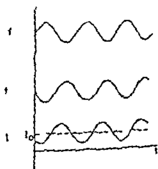


Fig 3 The relation between wave patterns … intensity f, Ordinary sinus wave f', second … a sinus wave, I theoretically deduced light … I_0, basic light intensity, t, time

presume that the mucous wave is approxi- by the formula

$$\xi_0 \sin \omega\left(t-\frac{x}{r}\right)$$

x position coordinate, t = time, ξ_0 = ude, ω = angle velocity, and r = velocity of ave The degree of curvature of a mathe- l function can be expressed by its second tive The second derivative of the function is obtained as

$$f''(x,t) = -\xi_0 \frac{\omega^2}{r^2} \sin \omega\left(t-\frac{x}{r}\right)$$

ssume that the recorded intensity varies n" to

$$+ \text{constant} \int_x^{x+\Delta x} f''(x,t)\,dx$$

I recorded intensity, I_0 = basic light ity, and Δx = the side of a square cor- to the inspected area in the micro- In reality the area is circular, but the difference is of little practical import- The following formula is obtained after uction and simplification

$$+ \text{constant} \sin \omega\left(t-\frac{x}{r}-\alpha\right) \sin -\beta$$

$\beta > 0$ for $\Delta x < \frac{\lambda}{2}$ where λ wavelength

that we get the same periodicity of the reflection as of the secretion wave This seen in Fig 3

RESULTS

rdings of mucociliary wave patterns from trachea are seen in Fig 4 For routine

4 Mucociliary wave patterns from the rabbit Recording speed 25 mm/sec (*above*) and sec (*below*) Frequency 12 waves sec

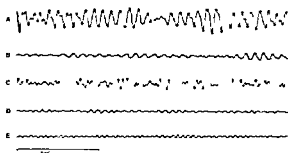


Fig 5 Mucociliary wave patterns from the rabbit trachea *A* Typical frequency and amplitude shifts. Mean frequency 12 waves sec *B*, Recording from the same place with a plastic film covering the area of observation. Mean frequency 15 waves sec *C* Recording from an untouched trachea Mean frequency 19 waves sec. *D*, Recording through a glass cover placed on the same place of the tracheal surface Mean frequency 15 waves sec. *E*, Recording through the glass cover after 15 min. Mean frequency 17 waves sec

purposes a low recording speed of 25 mm sec is used For frequency determination and for documentation the speed is raised to 100 mm/sec Amplitude and frequency variations are easily identified The mean frequency calculated during 30 sec on this record is 12 waves sec

Another record from the rabbit trachea is shown in Fig 5*A* Typical amplitude and frequency shifts are seen The frequency is 11–15 waves sec (mean value 12 waves sec) Fig 5*B* shows the effect when a small transparent plastic film is placed on the tracheal surface in front of the microscope The amplitudes of the light reflections are decreased and the wave frequency slightly increased (mean value 15 waves sec)

In the next experiment the initial mean frequency was 19 waves sec (Fig. 5*C*) A piece of glass measuring 15 1 5 mm and 0 15 mm thick, weighing about 0 1 mg was placed on the tracheal surface The amplitudes of the light reflections were reduced There was also a slight fall in frequency (mean value 15 waves/sec as seen in Fig. 5*D*) After about 15 min with the glass cover in place the amplitudes were still small but the frequency was somewhat increased (Fig 5*E*)

Simultaneous recordings from the tone gene-

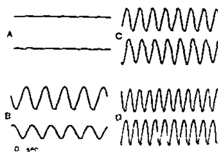


Fig 6 Simultaneous recordings from the tone generator output (*above* in *A B C* and *D*) and water surface waves recorded by the photomultiplier method (*below*) *A* Tone generator off *B* 10 Hz *C* 15 Hz, *D* 20 Hz

rator output and the reflections picked up by the described method from waves induced on a water surface are demonstrated in Fig 6 The upper oscillograms in *A*, *B*, *C* and *D* are obtained from the tone generator and the recordings below from the photomultiplier In Fig 6*A* the tone generator is off *B*, *C* and *D* illustrate comparisons at 10, 15 and 20 Hz respectively

The effect observed when a thin plastic film or a piece of glass is placed on the water surface is demonstrated in Fig 7 Three different frequencies of 10, 15 and 20 Hz have been used The recordings above are obtained directly from the tone generator output, those below from surface reflections via the photomultiplier In the xperiments shown to the left a polythene plastic)

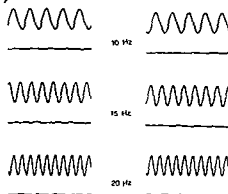


Fig 7 S multaneous recordings from the tone generator output and water surface waves recorded by the photo multiplier method To the *left* experiments with plastic film covering the area of reg stration To the *right* the same procedure with a thin glass covering Three fre quencies (10 15 and 20 Hz) are used The recordings *above* are from the tone generator output and the record ings *below* are from the photomultiplier

film covering is placed on the wa er reducing the waves In the record n st the water waves are eliminated by a t of glass

DISCUSSION

During the last four decades the cilian of the respiratory tract has been r studied with the use of objective me interest has previously been focuse transport velocity the ciliary beat f and the dynamics of ciliary movem Proetz, 1953, Rivera 1962) More rec medium in which the cilia function h s increasing attention (Barnett & M Sade et al , 1970) Today it is theref to speak about the *mucociliary sys* respiratory tract In cases of ineffectivet ation of secretions and particles the re be found either intracellularly in the p mechanism of the cell or extracellularl biorheological forces Consequently t mucociliary transport velocity and m wave movements ought to be used t ciliary transport and ciliary beat freq connection with direct and indirect i methods of ciliary activity in the r tract

In a previous paper (Mercke et al method is described for making objecti of the variations of light reflections about by cilia on an illuminated muc brane (Fig 4) In that paper it is als out that the reflections reproduced t movements within the mucous lay reflections may appear either on the s the mucous layer or on the tops of th of moving cilia Which theoretical an mental backgrounds can be quoted f two possibilities?

The mathematical deduction illus Figs 1–3 shows the general relations recorded surface reflections and wave m on a fluid surface The recordings of r from regular surface wave movements tonian fluids, e g water, are also in a with the calculations (Fig 6) The ca

ɔposed to be approximately valid for wave nents in mixed fluids like tracheo bronchial ons However, it cannot be excluded that f the incoming light is refracted through ɔn layer down to the carpet of moving In such a case a second type of wave ı must be taken into consideration

aim of this investigation has been to ıe proposed two origins of surface light ˌ The problem has been studied by rative experiments on ciliated mucous ranes and water surfaces Wave movements ı frequency are induced on a water ᵉ with the aid of a tone generator and a probe The waves are propagated in rings the surface, and the light reflected from ᵊves is recorded by the photomultiplier ɾd (Fig 6) This experiment shows the lity of the method for reproducing wave ˌ accurately These induced waves are ɟuced accurately for amplitudes and fre ıes The wave reflections obtained from bbit trachea, however, are irregular in these espects *Frequency- as well as amplitude* ions are always seen in the biological ıal (Fig 5*A*) Furthermore the irregular ions from the trachea may derive from at two reflection points—the mucous surface underlying carpet of cilia This is shown ᵎ following experiments

ıen a thin plastic film is placed on the ıus membrane in front of the microscope field the secretional waves are flattened ɜnd the reflection amplitudes decrease At ɜame *time the mean frequency increases* 5*B*) The light reflections are reduced but till possible to record the waves through the When the slightly heavier piece of glass ᵈ as a covering on the membrane, the ıtudes are further reduced The mean ᵉ is also reduced (Fig 5*D*) However, 15 min there is a frequency increase maybe biological response to a 'foreign body' on (Fig 5*E*)

hen the plastic film and piece of glass are in turn on a water surface, the ridges and ɿs are equalized so that all reflection variations disappear (Fig 7) In the corresponding biological experiments, however, reflections are still recordable (Fig 5) These results are therefore in accordance with our hypothesis that the light reflections emanate from the free ends of cilia as well as from the secretion layer

Biorheological model experiments on ciliary mucous membranes have been made by Miller (1966) and by Barnett & Miller (1966) These authors are of the opinion that the ciliary wave movements can be classified as "trochoidal" This means that the troughs are longer than the ridges They have also expressed the presumption that this difference could be the basis of secretion—and particle transportation The present authors have not, however, been able to verify this opinion Instead we are inclined to believe that amplitude and frequency shifts, which appear on the records, are due to biorheological forces of the mucus and bio chemical reactions within the ciliary cells This problem of the combined influence or individual factors listed above will be the object for further *investigations*

ZUSAMMENFASSUNG

Mittels einer standardisierten Mikroskop Photomultiplikator Technik sind in in vitro Experimenten die

konnte nachgewiesen werden dass sich die Oberflachen reflexe von respiratorischer Schleimhaut aus mindestens zwei Komponenten zusammensetzen und zwar dem *Reflex von der Schleimoberfläche* und dem vom Flim merhaarteppich unter der Oberfläche

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THE INFLUENCE OF TEMPERATURE ON MUCOCILIARY ACTIVITY

Temperature range 40°C–50°C

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act In vitro experiments have been performed on us membranes from rabbit trachea in order to igate the relationship between the mucociliary wave ency and an increase of temperature above body rature The following results have been obtained Optimum mucociliary activity was noted at or near ormal body temperature A further temperature rise 40° to 50 C did not noticeably influence the mucoy wave frequency
Mucociliary wave movements can be recorded dur-0 minutes at 49 C The critical temperature for irre-ble ciliestasis was estimated to be about 51°–52 C
The "mucociliary survival time" of mucociliary ac-in *in vitro* experiments decreased from about 5 hours C to about 1 hour at 49°C

previous paper (Mercke et al, 1974*b*) the ociliary wave frequency in the rabbit trachea been studied at temperatures between 20° 40°C An almost linear temperature/fre-ncy relationship was found in contrast to t biological activities, which usually increase mentially with increasing temperatures in range (Guttman, 1969)

he mucociliary activity of the respiratory t may be influenced and even destroyed at peratures above 40°C Febrile illnesses for nple are often complicated by an increased function and an insufficient elimination of etions It is therefore of theoretical as well as ractical interest to study mucociliary function n environment above the normal body temperature Only a few consistently performed experiments have hitherto been published regarding this problem

As early as in 1866 Roth observed that cilia from the respiratory tract of rabbits stopped beating at a temperature of 45 5°–46 5°C A-49 C the condition became irreversible The transportation rate of the mucociliary system was studied *in guinea pig trachea by Gordonoff* & Mauderli in 1936 These authors found an upper limit of transportation at 42 C The ciliary beat frequency was studied in *in vitro* experiments by Proetz (1934) and Iravani (1967) and their conclusions appear in Table I Proetz and Tanaka (1967) have studied the temperature influence on mucociliary activity *in vivo* However, their results differed a great deal and their methods were incomparable (Table I)

The duration of exposure must also be taken into consideration, for example in the case of applied research where mucociliary activity is chosen as an indicator of the effect of pharmaca and air pollution substances

The aim of the present study has therefore been

1 to analyse the relationship between mucociliary wave frequency and increasing temperatures in the temperature range 40°–50°C and

2 to find the maximum duration of exposure for maintained activity at a few temperature levels between 40° and 50°C in *in vitro* experiments

investigation has been supported by grants from the lish Medical Research Council, Project No B73 14X--01 and Project No B74-61P-4282-01

Table I

C – Cinematographical method S – Stroboscopical method P – Photoelectrical method

Authors	Method	Animal	*In vitro*	*In vivo*	Temperature for maximal frequency (°C)	Temperature for ciliostasis (°C)
Proetz, 1934	C	Rabbit		×	18–33	43–44
	C	Homo	×		18–33	43–44
Iravani, 1967	S	Rat	×		38–40	42–44
Tanaka, 1967	P	Rabbit		×	ca 30	—

METHOD AND MATERIALS

The standardized method for indirect recordings of the mucociliary activity via surface light reflections has been described in detail in a previous paper (Mercke et al, 1974*a*) Specimens of rabbit trachea mounted in a special experimental chamber (Mercke, 1974) have been used for *in vitro* experiments in an atmosphere above 90 % relative humidity and temperatures varying between 40° and 50°C.

Two experimental series have been carried out

1 Tracheal specimens from 9 rabbits have been exposed to continuously increasing temperatures The mucociliary wave frequency was recorded at 2° intervals between 40° and 50°C

2 Tracheal specimens from 25 rabbits have been exposed to constant temperatures of 40°, 42°, 45°, 47° and 49°C Five tracheae were used for each temperature level Recording was continued until no further surface activity could be observed in the microscope The duration ("mucociliary survival time") of retained activity was noted

RESULTS

1 *Rabbit tracheae exposed to increasing temperature*

The temperature of the specimen and of the surrounding air was continuously raised from 40° to 50°C during a mean time of 48 minutes (min 27 minutes, max 75 minutes) Fig 1 illustrates typical recordings from one of the nine experiments at six different temperatures The mucociliary wave frequency does not seem to change very much when calculated directly fr[om] series of recordings The results were als[o] sed by computer in order to find out [the fre]quency range Examples for one of the 40 [and] one of the 50°C levels are illustrated at F[ig] revealing no increase in wave frequency [with in]creasing temperatures The frequency ra[nge] about ±2 Hz in both cases

The wave frequency/temperature relat[ionship] was also plotted on diagrams which in tu[rn] processed by a computer A binomial re[gression] analysis for this relationship is shown [graphic]ally at Fig 3 The mucociliary wave fre[quency] differs very little from 40°C (1060 waves/min) to 50°C (1130 waves/min) The amplitude fre[quency] pattern *maintains roughly* the periodical rhythm during the whole tempe[rature] range (Fig 1)

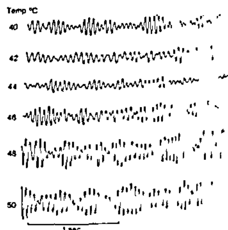
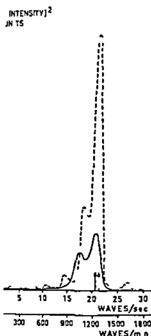


Fig 1 Mucociliary wave frequencies recorded [from] tracheal specimen at different temperatures [be]tween 40° and 50 C



2 The relationship between mucociliary wave fre-ty at 40°C (continuous line) and 50°C (broken line) reflected light intensity in the sequence also shown g 1

Rabbit tracheae exposed to constant tempera-ures

heal specimens from 25 rabbits were exposed onstant temperatures of 40°, 42°, 45°, 47° and C respectively At each level 5 rabbits were mined The duration of mucociliary function own in Fig 4 The duration of the activity eased with increasing temperatures There a statistically significant difference between 40° and the 49°C "mucociliary survival time" lcoxon rank-sum-test, $p<0.01$)

Vave frequency was sampled at 10 minute rvals for the 40°, 42° and 45°C levels and at inute intervals for the 47° and 49°C levels results are seen in Table II

regression line for each temperature level calculated by computer The results appear ig 5 The duration is only for the period in ch at least 3 out of 5 specimens showed any vity at all It will be noticed that the initial ociliary wave frequency at every temperature l is concentrated within a small range (1060 00 waves/min)

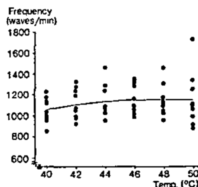


Fig 3 Binomial regression line for all wave frequencies and temperatures in the range 40°–50 C from 9 animals

With increasing exposure time in constant temperature experiments, the mean frequency decreases very slowly at all levels except the 47 C level It is also obvious that the slopes of the regression lines are very similar and nearly horizontal

DISCUSSION

The relationship between mucociliary activity and temperatures from 40° to 50°C has been investigated in order to determine (1) the temperature range for maximum activity, (2) the maximum temperature for maintenance of any activity at all, and (3) the "mucociliary survival time" for tracheal specimens at different temperature levels in *in vitro* experiments The same method of investigation has previously been used

Temp	Survival time	Mean value (minutes)
49 °C		60
47 °C		112
45 °C		133
42 °C		258
40 °C		307
	60 120 180 240 300 360 420 min	

Fig 4 The relationship between mucociliary survival time (the time during which activity can be recorded from the mucous membrane) and different temperatures in the range 40°–49°C.

Table II

40° C		42° C		45° C		47° C		49 C	
Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency [illegible]
12	978	10	886	10	934	10	1 217	10	[illegible]
22	1 108	20	930	19	838	15	1 096	15	[illegible]
32	1 004	30	877	28	964	20	1 144	20	[illegible]
43	1 020	40	919	38	1 103	25	1 212	25	[illegible]
53	969	50	1 072	48	1 086	30	1 231	30	[illegible]
63	942	60	947	58	1 034	35	1 229	35	[illegible]
73	1 094	70	1 016	68	950	40	1 204	40	[illegible]
83	1 026	80	1 104	78	942	45	1 102	45	[illegible]
93	994	90	1 021	88	904	50	1 133	50	[illegible]
103	920	100	998	98	918	55	1 135	55	[illegible]
113	978	110	924			60	1 210		
123	1 041	120	1 004			65	1 187		
134	950	130	1 028			70	1 126		
144	1 080	140	1 175			75	1 233		
153	1 072	150	995			80	1 207		
163	1 025	160	813			85	1 298		
173	980	170	809			90	1 199		
183	956	180	825			95	1 170		
193	971	190	813			100	1 330		
203	929	200	675						
213	930	210	885						
223	948	220	846						
233	969	230	880						
243	882	240	796						
253	1 218								
263	1 049								
273	1 085								
283	959								
293	930								
303	851								
313	884								
323	837								
333	914								

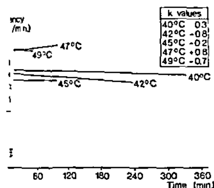
analogous experiments in the temperature range of 20° to 40 C (Mercke et al, 1974*b*)

The temperature borderline between the two investigations is just above the body temperature of rabbits, which is 37°–39°C according to Dalhamn (1960) and Altman & Dittmer (1966)

Between 20° and 30°C there was a mucociliary wave frequency increase of 300 waves/min which means a Q_{10} value of 2 1 In the next 10°C range the frequency increase was somewhat smaller i e a Q_{10} value of 1 4 The present results show no further frequency increase following a continuous temperature rise to 50°C As seen in Fig 3 there was no mucociliary frequency difference from 40 C (1060 waves/min) to 48°C (1 140 waves/min) The Q_{10} value of this range was 1 1

This means that the mucociliary wave [illegible] have already reached their maximum at [illegible] body temperature and that a further temper[illegible] increase from 40° to 50 C has hardly [illegible] additional influence on this activity No d[illegible] temperature peak for maximum activ[illegible] be seen Even the mucociliary wave pa[illegible] mained unchanged during the temper[illegible] crease (Fig 1) However, the validity of [illegible] conclusions must be confirmed in *in vivo* [illegible] ments

Below body temperature the wave fre[illegible] is influenced by two factors One is the [illegible] cellular "pacemaker" rhythm Another [illegible] rheological property of the mucus (T[illegible] et al, 1974) Both these parameters [illegible]



Regression lines for five different temperatures in [ra]nge 40°-49°C The lines represent that part of the [experi]ment in which at least 3 out of 5 tracheal specimens still alive

as a result of temperature changes The [...]logical factor—which is said to decrease [...]rly with increasing temperatures (Adamson, [...])—is very important at low temperatures obviously has less influence above 40°C even the intracellular activity seems to be [...]ulated above body temperature but this [inter]esting observation ought to be tested with [reco]rdings of the intracellular electrical activity [(J]ansson & Toremalm, 1966) at varying [tem]peratures

[In] the series of constant temperature experi[men]ts there is an initial increase in wave fre[quen]cy at 42° and 45°C

[In] the other series, however, the wave fre[quen]cy is continuously high until the activity [sud]denly disappears (Table II) The reduced [mu]cociliary survival time" of the tracheal [spec]imens *in vitro* at high temperatures may be [due] to changes of the biochemical processes

[W]hat is the temperature level for irreversible [muc]ociliary activity? Proetz (1934) found such [a m]aximum temperature at 43°-44°C in rabbits [usi]ng *in vivo* experiments and the same tempera[ture] range *in vitro* in human tissues However, [the] humidity factor was not sufficiently con[tro]lled in these experiments Iravani (1967) is [of t]he opinion that 42°-44°C should be looked [upo]n as the critical temperature If an extra[pola]tion is made from the present results (Fig [5]) the critical temperature appears to be at [abo]ut 51°-52°C

A regression line has been calculated by a computer (Fig 5) for each temperature on the basis of the mean frequencies for each measuring interval The regression lines are practically parallel and almost horizontal This means that the initial mucociliary wave frequency is maintained over the entire range from 40° to 49 C without any distinct frequency peak

The present results are of little interest from a clinical point of view However, the constant temperature experiments can be valuable for applied experimental purposes because the maximum duration of such experiments should not exceed 5 hours at 40°C, 4 hours at 42°C, 2 hours at 45°C and 47°C and 1 hour at 49°C

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I am greatly indebted to Daniel Huberman, B Sc, for valuable laboratory assistance

ZUSAMMENFASSUNG

Bei *in vitro* Versuchen an der Trachealschleimhaut von Kaninchen ist die Abhängigkeit der mukoziliaren Wellen frequenz von Temperatursteigerungen uber Korpertemperaturniveau untersucht worden Die Versuche ergaben folgende Resultate

1 Optimale mukoziliare Aktivitat erfolgte bei oder nahe bei normaler Korpertemperatur Eine Erhohung der Temperatur von 40 C auf 50 C hatte keinen fest stellbaren Einfluss auf die mukoziliare Wellenfrequenz.

2 Mukoziliare Wellenbewegungen konnen bei 49 C während 60 Minuten registriert werden Die kritische Temperatur fur irreversibles Erloschen der Flimmer aktivität wird auf ungefahr 51 C-52 C geschatzt

3 Die mukoziliare Überlebenszeit bei *in vitro* Versuchen sank von ungefahr 5 Stunden bei 40 C auf zirka eine Stunde bei 49 C

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PLETHYSMOGRAPHIC STUDIES OF THE BLOOD FLOW IN THE MUCOSA OF THE HUMAN MAXILLARY SINUS

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act Plethysmography of the mucosa of the maxillary has been possible due to following facts and obser ns
The maxillary sinus is an air filled cavity with com ly rigid walls
Its ostium can be completely blocked experiment

The surface area of the mucosa in the maxillary can be calculated roentgenographically
Pulse waves are sometimes seen during pressure re- ngs from maxillary sinuses with obstructed ostium
Similar pulse waves are almost always present in al sinuses with experimentally closed ostium.
An upward slope of the pulse wave recording is ned during compression of the jugular veins
All errors involved in such plethysmographic re ings seem to result in an underestimation rather than verestimation of the blood flow The mean blood obtained by measurements during bilateral jugular pression was 0 26 μl/cm² sec With an estimated osal thickness of 125 μm the blood flow will be 125 00 cm³ min. This would mean that the blood flow e maxillary mucosa is greater than in muscle brain liver and only surpassed by the kidney and lungs duction of mucosal thickness and of the pulse waves tained during compression of the carotid artery

blood flow of the mucous membranes in upper respiratory tract is influenced by differ- stimuli of an emotional, thermal, or physical re It has also been proposed that there ts an abnormal mode of blood flow and od vessel reaction in some nasal diseases, ifically vasomotor rhinitis (Godin, 1961, lstedt & Rundcrantz, 1964) Animal experi- its have shown that the blood flow of the al mucosa changes in different ways in re- nse to different stimuli (Naumann, 1961)

work was supported by the Swedish Medical Re- h Council (project 749)

Animal experiments with qualitative studies of the blood flow have contributed considerably to our present knowledge of the blood flow of the respiratory mucosa

To obtain a more exact understanding of the blood flow of the mucosa in man it is necessary to have quantitative methods for studying the blood flow Photoelectric plethysmographic stud- ies of the nasal mucosa (Davis & Hertzman, 1957, Kato, 1965, Yamakawa, 1967) have given valuable information on the blood flow but so far only qualitative studies have been published

Thermal conductivity measurements of the nasal blood flow have been performed as semi- quantitative *measurements (Drettner, 1961,* Schroeteler, 1964) However, no quantitative measurements of the human mucosal blood flow in the upper respiratory tract, nor in any other human mucosa in vivo, have been published This lack stands in contrast to the multitude of reports of quantitative blood flow studies in the skin, muscle, etc The lack of mucosal blood flow studies is obviously a consequence of the difficulties encountered in devising methods for such investigations

The purpose of this work is to present pre- liminary results of a new method for the quantita- tive study of blood flow of the maxillary sinus mucosa

The blood flow of the mucosa of the paranasal sinuses deserves to be studied since the mucosal swelling—well known in several diseases of an infectious, allergic, or vasomotor nature—must to some extent be connected with blood flow

changes Furthermore all calculations of the gas exchange in the paranasal sinuses will be based on a more solid ground if they are combined with blood flow measurements in the mucosa

THEORETICAL ASPECTS

The maxillary sinus, and to some extent also the frontal sinus, has a unique property for plethysmographic blood flow studies in comparison with other organs containing a mucosa, namely a cavity with completely rigid walls except for the ostium which is small and can be obstructed experimentally or spontaneously Furthermore, it has a mucosa of which the surface area can be calculated In order to study the patency of the maxillary ostium the pressure within the maxillary sinus is often recorded and during such recordings pulse waves are sometimes seen in the recordings In almost all healthy sinuses with experimentally obstructed ostium this pulse wave was seen Such pulse waves were described by Drettner (1965) and they were observed occasionally in patients with total or partially obstructed ostia, though not in those with patent ostia

Recording of these pulse waves can be used for plethysmographic measurements if the blood flow from the sinus can be essentially stopped for a few seconds and if the volume of the sinus [illegible] be measured A method for the latter has [illegible] elaborated (Aust & Helmius 1974) Furthermore, it is necessary to have information about the ostial patency, which is easily available (Drettner, 1965)

The blood vessel to the maxillary sinus belongs, on the arterial side, principally to the external carotid system and on the venous side to the internal and external jugular systems, while the vertebral arterial and venous systems do not supply the mucosa (Wagemann, 1964) In that way blockage of the arterial or venous supply to the mucosa will be possible by compression on the neck but some blood flow to or from the vertebral system or other non blocked systems, cannot be completely avoided The short interval—only a few seconds—during which the blockage is performed will probably [illegible] arterial supply or venous outflow [illegible] than the ordinary ways

MATERIAL AND METHODS

Ten persons (age range 20 to 63 [illegible] investigated All of these subjects were [illegible] with normal rhinoscopy and normal [illegible] grams of the paranasal sinuses.

The investigation was performed [illegible] subject in a semirecumbent position. The [illegible] sure within the maxillary sinus was reco[illegible] introduction of a cannula with a side [illegible] the sinus The instruments used were a [illegible] transducer (EMT 33, Elema Schönander [illegible] holm), an amplifier (EMT 31) and a [illegible] (Mingograph 34) The obstruction of the [illegible] caused by swollen mucosa or by a tampo[illegible] checked by lowering and raising the [illegible] (about 5 cmH_2O) in the sinus and [illegible] unit with a syringe connected to the ma[illegible] The volume of the sinus was measu[illegible] roentgenographic method (Aust & H[illegible] 1974)

Compression of the carotid artery [illegible] formed manually, using two fingers and [illegible] strong pressure The internal and external [illegible] lar veins were compressed together [illegible] somewhat more lateral, yet not so force[illegible] pression A few trials were usually necess[illegible] fore a selective and effective compressio[illegible] obtained All compressions were perform[illegible] ruptly and lasted only a few seconds. Thre[illegible] iments were performed and the mean of th[illegible] used

In order to calculate the blood flo[illegible] surface area it was necessary to ca[illegible] the surface of the mucosa of the ma[illegible] sinus This was done by using the para[illegible] obtained during roentgenographic measu[illegible] of the sinus volume A mucosa which [illegible] (or only a slight) swelling seems to be [illegible] mandatory for the calculation of the s[illegible] area (Aust & Helmius 1974)

Information on the patency of the ma[illegible] ostium was obtained by simultaneous [illegible]

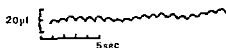
·I

·t	Age	Ostial patency	Sinus volume Roentgen ologically (ml)	Mucosal surface (cm²)
	20	Patent	10 26	36 60
	28	Patent	22.22	63 54
	25	Patent	17 69	48.23
	26	Patent	15 23	45 68
	27	Patent	13 04	41 17
	20	Patent	16 38	48 70
	63	Patent	13 40	40 70
	20	Patent	16 56	47 94
	27	Patent	12.29	41 41
	34	Patent after blowing	14 93	43 95
			15 20	45 79

-dings in the maxillary sinus and nasal cavity ıg breathing, blowing and sniffing (Drett- 1965)

RESULTS

e I shows that the maxillary ostium was ılly patent in 9 of the subjects and become nt after blowing of the nose in the last sub- The volume of the maxillary sinus measured tgenographically is given from Table I The ıce area of the mucosa in the maxillary sinus ılated according to a previously published ıula is also presented in that table An ɑple of a pulse wave recorded from the ıllary sinus is shown in Fig 1 The magni- of the pulse waves in all 10 subjects corre- ıded to a mean volume increase of 8 2 µl/beat ¹7 µl/sec This represents the blood added



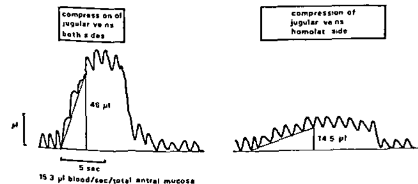
5 µl blood/heart beat/total antral mucosa
0 1 µl / /cm2
1 µ increase of mucosal thickness/heart beat

Fig 1 Pressure variations in the maxillary sinus due to pulse waves in the vessels of the antral mucosa in a healthy maxillary sinus with an experimentally closed ostium Calculations of the increase in blood flow due to these pulse waves

to the vessels by the systolic action of the heart while the basal blood flow is not taken into account

When the jugular veins were compressed on both sides the slope of the plethysmographic recording could be used for blood flow measurements (Fig 2) The mean increase measured for the total antral mucosa was 11 6 µl/sec during bilateral jugular compression ($n=7$) and 7 5 µl/sec during ipsilateral jugular compression ($n=7$), while contralateral jugular compression gave a mean increase of 1 8 µl/sec ($n=7$) When the value measured during bilateral jugular compression is correlated with the mucosal surface area, a mean of 0 26 µl/cm² sec is obtained The thickness of the mucosa of the sinuses is reported in the literature (Loring & Tenney, 1973) to be 125 µm A blood flow of 20 9 µl/cm³ sec is thus obtained for the mucosa or 125 ml/100 cm³ min

The maximal increase of the antral mucosa during a Valsalva manoeuvre was also measured



2 Pressure rise in the maxillary sinus at bi- and uni ·al compression of the jugular veins



Fig 3 Pressure rise with intact pulse waves in the maxillary sinus during Valsalva manoeuvre

(Fig 3) The mean increase was 25 2 µl for the antral mucosa in 5 subjects, or 0 58 µl/cm² When using a value of 125 µm for the thickness of the antral mucosa an increase of 46 4 µl/cm³ was obtained

Plethysmographic recording during compression of the common carotid artery of the same side was performed on 2 subjects (Fig 4) A vanishing of the pulse wave and a downward slope of the plethysmographic recording was obtained The mean decrease in volume of the total antral mucosa was 14 1 µl, or 0 26 µl/cm³

DISCUSSION

This appears to be first quantitative measurement of the blood flow of any mucosa in man However, the magnitude of the error due to incomplete interruption of all blood flow from the sinus during the jugular compression cannot be evaluated An outflow through the vertebral or other non-blocked veins cannot be completely [illegible] but was probably small when the jugular compression lasted only a few seconds It is also impossible to be sure that all blood flow in the jugular veins was blocked during the compression These errors work in the same direction, implying that the values of blood flow obtained during bilateral jugular compression are probably too low There can hardly be any



Fig 4 Pulse wave recording from the maxillary sinus during compression of the carotid artery on the homolateral side Pronounced reduction of the waves and a decreased pressure level in the sinus indicating reduction of the thickness of the mucosa

Table II. *Results of antral plethysmography [illegible] ing jugular compression*

Case no	Slope during jugular compression: Contralateral compression (µl sec⁻¹)	Homolateral compression (µl sec⁻¹)	Bilateral compression (µl sec⁻¹)	[illegible]
1	0	1 735	—	—
2	0	4 050	4 725	[illegible]
3	0	11 330	11 590	[illegible]
4	—	—	—	—
5	3 17	5 433	5 890	[illegible]
6	2.15	4 300	10 010	[illegible]
7	4 63	3 700	20 350	[illegible]
8	—	—	—	—
9	2 61	13 020	13 560	[illegible]
10	—	12 950	14 980	[illegible]
Mean	1 79	7 460	11 570	[illegible]

error of importance in the opposite [illegible] caused by these factors The compressib[illegible] the air in the sinus, tubes of the [illegible] system and pressure transducer is a factor [illegible] may cause an error in the recordings. [illegible] as in all plethysmographic studies with [illegible] systems this factor is already consid[illegible] the calibration (Graf & Westersten [illegible] an error of importance only occurs d[illegible] rapid pressure changes Furthermore [illegible] will therefore only give an underestima[illegible] an overestimation, of the blood flow

A back flow of the blood might theoretica[illegible] possible since the veins have no valves. [illegible] the only blood which may flow backw[illegible] that compressed by the fingers during the [illegible] compression

Table III *The blood flow in some human [illegible] according to* Textbook of Physiology [illegible] chemistry (1959) *and* Handbook of R[illegible] (1958) *The value of the mucosa of the [illegible] sinus is obtained from the present [illegible]*

Tissue	Blood flow (ml 100 g⁻¹ tissue [illegible])
Muscle, forearm, rest	2
Muscle, forearm, exercise	30
Brain	65
Liver	100
Mucosa, maxillary sinus	125
Kidney	400
Lungs	450

1 overflow from other organs in the head occur but it must be small during a meas- period of 3 seconds especially since the s have a parallelly and not a serialy con d circulation In roentgenographic inves ons of the blood flow in the neuro cranium is never any overflow to the vessels of the ro cranial organs even when the jugular veins ompressed (Bergstrom 1974)

may be of interest to compare the results e blood flow obtained in the mucosa of the llary sinus with values of blood flow in organs Table III shows reports from the ture concerning the blood flow in different s Using the approximation that a 100 cm³ al mucosa has a weight of 100 g the blood of the antral mucosa is 60 times that of ng muscle, 4 times that of active muscle, that of the brain, slightly more than that e liver, while the kidney and the lungs have ater blood flow than the antral mucosa

e antral mucosa therefore must be consid to have a large blood flow, which contradicts assumption of Loring & Tenney (1973)

seems likely that the present method can sed in experimental blood flow studies, for nple in pharmacological or similar experi ts

ZUSAMMENFASSUNG

ysmograph e der Kieferhohle ist bei folgenden Tat n und Beobachtungen moglich

D e Kieferhohle ist ein luftgefullter Hohlraum mit and g ste fen Wanden

Ihr Ostium kann exp rimentell vollständig blockiert en

Die Oberflache der Kieferhohlenschleimhaut kann enograph sch berechnet werden

Pulswellen sind manchmal wahrend Druckregistrie en von der Kieferhohle bei verschlossenem Ostium hen.

Ähnl che Pulswellen sind fast immer in normalen rhohlen mit tamponiertem Ostium vorhanden

Ein Anste gen der Pulswellenregistrierung wird wah Kompression der Jugularvenen erreicht

Alle Fehler mit denen solche plethysmographischen str erungen behaftet sind *scheinen eher eine Unter* ttung und wahrscheinlich keine Überbewertung des flusses zu ergeben Der durchschnittl che Blutfluss bei Messungen wahrend der Kompression auf die erse t gen Jugularvenen erreicht wurde war 0 26 n sec Bei einer geschätzten Schle mhautdicke von 125 μm ergibt sich ein Blutfluss von 125ml/100 cm³ min Das wurde bedeuten dass der Blutfluss in der Kiefer hohlenschleimhaut grosser ist als im Muskel im Gehirn und in der Leber und nur von dem in der Niere und der Lunge ubertroffen wird Eine Reduktion der Schleimhaut dicke und der Pulswellen wird bei einer Kompression auf die A Carotis communis erreicht

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OXYGEN TENSION IN THE HUMAN MAXILLARY SINUS UNDER NORMAL AND PATHOLOGICAL CONDITIONS

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Abstract The pO_2 in the maxillary sinus measured with a small pO_2 electrode was found primarily to be dependent on the patency of the maxillary ostium It was also correlated with the functional size of the maxillary ostium when this was smaller than 2 5 mm in diameter A mean oxygen tension of 116 mmHg was found in the maxillary sinus of normal cases and a mean antral pO_2 of 75 mmHg in patients with sinusitis The pO_2 could not be shown to be related to the presence or absence of antral pus or mucus in the cases with sinusitis However, oxygen tensions of only a few mmHg were found in two cases with aerobic bacteria in the antral pus Pain in the region of the paranasal sinuses was to some extent correlated to low antral pO_2

Change in the oxygen content in the paranasal sinuses after obstruction of the ostium has been associated with the pathophysiology of sinusitis and vacuum sinus (Sluder, 1927, Flottes et al, 1960)

A few experiments concerning oxygen changes in paranasal sinuses after ostial obstruction have been reported in the literature A decreasing oxygen (and increasing carbon dioxide) tension in the frontal sinus of dogs was found by Doiteau (1955) after occlusion of the naso frontal duct Studies in man with Scholander's method of aspirated air from the maxillary sinus showed a slightly lower oxygen tension in patients with sinusitis than in normal subjects, 16 60 % and 17 29 %, respectively (Kitayama, 1968) Aust & Drettner (1971) introduced a small pO_2 electrode into the sinus in man and found, in a preliminary report, that the antral oxygen tension was correlated to the patency of the maxillary ostium

This work was supported by the Swedish Medical Research Council (project 749)

The purpose of the present investigation [illegible] analyse the oxygen tension in the [illegible] sinus in relation to the clinical d[illegible] sidering in particular the patency of the [illegible] ostium, the functional size of the [illegible] *volume of the sinus, the presence or [illegible]* mucus in the sinus, recurrent sinusitis [illegible] occurrence of pain in the forehead or [illegible]

MATERIAL AND METHOD

The series consists of 97 persons on [illegible] investigations were performed (Table 1 [illegible] subjects were investigated twice

The oxygen tension in the sinus was [illegible] with a small pO_2 electrode (Brotzu & [illegible] 1967, Aust & Drettner, 1972) introduc[illegible] the maxillary sinus through the inferior meatus after anesthesia with Xylocain (Astra) Calibration was performed acc[illegible] a method previously reported (Aust & D[illegible] 1972), with the exception that a temper[illegible] 36 0°C was used instead of 37 0 C, [illegible] perature measurements with thermo[illegible] the human maxillary sinus showed a temperature of 36 0 C

The patency of the maxillary ost[illegible] investigated with simultaneous pressure [illegible] ings in the sinus and the nasal cavity [illegible] breathing blowing and sniffing (Drettner [illegible] The volume of the sinus was measured [illegible] genolographically (Aust & Helmius, 19[illegible] functional size of the maxillary ost[illegible]

e I *Presentation of the material*

nosis	Number of subjects	Number of investigations
al subjects	32	38
eostomies	3	3
tio septi nasi	9	9
notor rhinitis	10	11
polyps	7	8
sinusitis	15	16
sinusitis tal)	5	6
rent acute sinusitis ve period)	5	5
rrent acute sinusitis nt period)	6	6
s	5	5

mined by pressure recordings in the maxil s sinus during momentary air insufflation a known airflow (Aust & Drettner, 1974)

RESULTS

nosis

e II shows the oxygen tension in the different nostic categories

s seen in the table the average oxygen tension e maxillary sinus of normal subjects was 4 mmHg or 16 3 %. The group with nasal

le II pO_2 *in the maxillary sinus in relation to nosis*

nosis	Number of investigations	Mean pO_2 mmHg	Mean pO_2 %	S D mmHg
nal subjects	38	116 4	16 3	23 9
nal with wn patent um	32	117 6	16 5	32 6
heostomies	3	90 3	12 7	9 3
atio septi i	9	105 6	14 7	40 8
motor rhini	11	106 5	14 9	41 4
l polyps	8	79 5	11 2	28 5
e sinusitis	16	74 8	10 5	48 7
e sinusitis tal)	6	74 9	10 5	30 1
rrent acute sitis (acti period)	5	101 6	14 2	65 4
rrent acute sitis (silent iod)	6	99 0	13 9	40.3
rs	5	140 5	19 7	23 4

disorders such as deviatio septi nasi and vasomotor rhinitis had a slightly lower pO_2

The patients with acute sinusitis had a mean value of about 75 mmHg, with no distinction between dental and non dental sinusitis The group with recurrent sinusitis had a mean oxygen tension of about 100 mmHg (14 0 %) with no difference when measured in the silent or active period A silent period is defined as an interval of at least 3 months during which the patient with recurrent sinusitis has been free from any acute exacerbations

Nasal breathing

In 3 patients without any nasal breathing but normal anatomy, i e 2 patients who had undergone laryngectomy and one patient with a tracheostomy, the mean pO_2 in the maxillary sinus was found to be 90 3 mmHg (12 7 %)

Ostial patency

The antral oxygen tension in relation to the patency of the maxillary ostium is shown in Table III

The mean value with a patent ostium was 116 5 mmHg (16 3 %) and for a partially patent ostium, 100 0 mmHg (13 6 %) The cases with ostia functioning as valves had a mean value of 99 8 mmHg (14 0 %) and those in which the ostia were obstructed even during blowing/sniffing had a mean value of 80 7 mmHg (11 3 %) Cases in which patency was achieved after blowing and sniffing were too few for any conclusive results It is interesting to note that there were no antral pO_2 s below 30 mmHg in the groups with patent, partially patent, patent after blowing/sniffing or valvular ostia, while 5 patients with obstructed ostia had oxygen tensions below 30 mmHg and 4 of these were below 10 mmHg

Functional ostial size

The functional size of the maxillary ostium was measured in 24 normal sinuses in which pO_2 was determined simultaneously The mean pO_2 of this group was 128 5 mmHg (18 0 %) There was a positive correlation between the oxygen tension in the maxillary sinus and the size of the

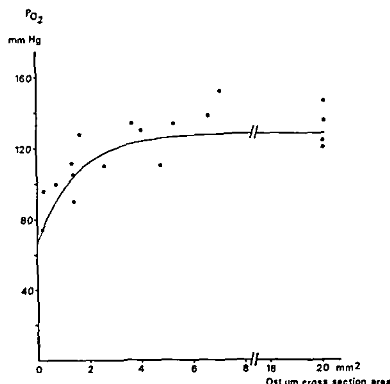


Fig 1 Relationship between the [illegible] maxillary sinus and the [illegible] the maxillary ostium. The [illegible] the relationship is a [illegible] a correlation coefficient of [illegible] measured datas.

ostium up to a cross sectional area of about 5 mm² or a diameter of about 2 5 mm (Fig 1) The line depicting the relationship between ostial size and antral pO_2 in Fig 1 was mathematically calculated and has a correlation coefficient of 0 88 In addition it shows that the antral pO_2 is constant in sinuses with ostial cross sectional areas larger than 5 mm²

Antral volume

The volume of the maxillary sinus calculated from roentgenograms (Aust & Helmius, 1974) was measured in 27 healthy persons in whom antral pO_2 also was determined In addition in 18 of these persons the functional size of the maxillary ostium was measured manometrically Analyses of the data from the examined group revealed no correlation between the pO_2 in the sinus and the antral volume

Cases of Sinusitis

Presence or absence of mucus in the sinus

In 14 of the 16 patients with non dental sinusitis, antral washing was performed with saline according to ordinary therapeutic methods. [illegible] patients had purulent contents in the [illegible] 7 patients had no antral pus The [illegible] sinuses with purulent contents was [illegible] (10 5 %) while patients without [illegible] a mean pO_2 of 61 mmHg (8 6 %). [illegible] ence was not statistically [illegible] cases with purulent content had a [illegible] metric pressure indicated by [illegible] flow of pus through the cannula [illegible] washing In a few cases the oxygen [illegible]

Table III *pO_2 in the maxillary sinus* [illegible] *to the patency of the maxillary ostium*

Ostial status	Number of investigations	Mean pO_2 mmHg	Mean pO_2 %
Patent	46	116.5	143
Partially patent	5	100 0	134
Patent after blowing or sniffing	3	80 7	113
Valve acting	13	99 5	130
Obstructed	27	80.7	113
Not investigated	13	93 8	112

IV *Relationship between ostial status and l pO_2 in cases with recurrent sinusitis in and silent period*

period			Silent period		
	pO_2		Ostial status	pO_2	
	mmHg	%		mmHg	%
	92	12 9	Patent	154	21 6
	90	12 7	Patent	145	20 3
g	160		Partially patent	69	9 7
ded	163		Partially patent	65	9 1
ded	3	0 4	Valve acting	70	9 8
			Occluded	91	12 8

times very high, as seen in 2 cases in Table or example The oxygen percentage in these was not known as the manometric pressure not measured

riology

n patients with inflammation and pus or is in the maxillary sinus had positive bac l cultures taken from the antral washings e antral washing was classified with (–) no discharge and (+), (+ +), (+ + +) for according to amount, colour and viscosity e V shows the diagnosis, ostial status, bac- and pO_2 in the investigated sinuses In this it is obvious that most maxillary sinuses with an abundant purulent content usually had a lower pO_2 than a sinus with less However, one patient with (+ + +) in antral washing and patent ostium had a high antral oxygen tension

In the table we can also see that there is a tendency to higher pO_2 in the sinuses with more patent ostia than in those with occluded ostia

Two patients with very low pO_2 values, 5 and 6 mmHg, and (+ + +) in antral washings had purulent infections with aerobic bacteria Two others had (+ + +) in discharge but antral pO_2 of 65 and 84 mmHg These patients had both dental sinusitis with anaerobic bacteria However, only one of them had a completely occluded maxillary ostium

Pain

In the series of patients with sinusitis, 9 patients had pain in the forehead or over the maxillary sinus The severity of the pain was not investigated, nor was the manometric pressure in the sinus measured

Pain in the forehead and sinuses can occur at greatly differing antral pO_2 levels, but it seems to be most prevalent in cases with a low antral pO_2 values (Table VI)

DISCUSSION

This investigation is the first in which the oxygen tension in the maxillary sinus has been analysed

le V *Findings in 11 patients with positive bacterial cultures*

nos s	Ostial status	pO_2 mmHg	pO_2 %	Antral washing	Bacteria
it s acuta	Occluded	32	4 5		*Staphylococcus aureus*
itis acuta	Occluded	62	8 7	–	*Staphylococcus aureus*
itis acuta	Valve acting	92	12 9	+	*Staphylococcus aureus*
itis recidivans te exacerbation	Patent	147	20 6	– –	*Staphylococcus aureus*
situs acuta	Occluded	6	0 8	+	*Haemophilus influen ae*
situs acuta	Partially occluded	128	17 6	+	*Haemophilus influen ae*
itis acuta	*Occluded*	*5*	*0 7*	–	*Flavum bacteria*
situs acuta	Occluded	134	18 8	+	*Staphylococcus albus*
situs acuta dental	Occluded	51 5	7 2	–	Pyoceaneus
situs acuta dental	Partially occluded	65	9 1	+ + +	Gram pos anaerobic coccus
sit s acuta dental	Occluded	84	11 8	+ + +	Gram pos anaerobic coccus

Table VI *pO_2 in relation to pain in 9 patients with sinusitis*

Ostial status	Discharge	pO_2 mmHg	%
Occluded	−	3	0 4
Occluded dental fistula	+ + +	52	7 3
Valve acting	+	57	8 0
Occluded	+	69	9 8
Occluded	−	75	10 5
Patent	−	75	10 5
Occluded	−	80	11 2
Occluded	+	128	18 0

in a large series of persons with a method avoiding most of the problems of previously used techniques Some technical problems have been encountered, however Whilst introducing the cannula with the small pO_2 electrode through the bony wall of the lower nasal meatus into the sinus, the electrode membrane sometimes varied its position, thus altering the calibration A new calibration was therefore performed after each experiment and if the values differed by more than 20 mmHg, the experiment was abandoned A difference of 20 mmHg was accepted since a small drift could occur during the hour s time usually required (Aust & Drettner, 1972) for these measurements

The mean antral oxygen tension in all normal subjects was 116 4 mmHg (16 3 %) while in those where it also had been shown that the maxillary ostium was patent, 117 6 mmHg (16 5 %)

Flottes et al (1960) reported an oxygen content of 17 5 % in the frontal sinus of normal dogs, which is almost identical with our measurements in living man

Two factors can be thought to cause a decrease in the oxygen tension of the maxillary sinuses, namely the low pO_2 of the expiratory air and the absorption of oxygen in the antral mucosa The absorption in the mucosa can be counteracted by adequate ventilation through the ostium requiring a patent ostium This ventilation is also influenced by the nasal respiration (Aust & Drettner, 1971)

Patients without nasal breathing, but with normal nasal anatomy and patent os... investigation 3 persons who had ... tracheostomy or laryngectomy) had ... pO_2 which was lower than in normal ... probably due to the lack of nasal resp... consequently a reduced ventilation th... ostium The number of patients w... respiration was, however, too small f... conclusions

The investigation showed that the... tive correlation between the antral pO... functional size of the ostium up to ... sectional area of 5 mm² (ostium dia... mm) In maxillary sinuses with still l... the pO_2 was found to be about 1... regardless of ostial size which implies ... sinus is sufficiently ventilated to cou... oxygen absorption in the sinus In s... smaller ostia, the pO_2 is lower and ... to the functional size of the ostium ... polation to zero of the ostium the pO_2 ... 70 mmHg (Fig 1) This value is n... from the mean value found in pa... obstructed ostia, 80 7 mmHg

A relative insufficiency of antral ve... thus present at ostial diameters less th...

It seems likely that factors which ... of relative small importance for an... tion, e g decrease in nasal breathing ... the actual side of the nose, for example ... deviations or variations caused by ... cycle, can be of importance for the ... when the ostium is small

Patients with sinusitis had a low a... compared with normal subjects The... difference in patients with non-dental ... infections Neither was there any ... difference in cases with or without pa... in the sinus The pO_2 in the sinus ... pally related to the patency of the os...

Patients with recurrent acute s... both in active and in silent periods ... pO_2 which was principally corre... patency of the ostium In both the pa... acute exacerbations of the recurrent ... and in those in the silent period ... found both patent and occluded os...

representation in both groups of non-patent

A correlation between disturbed patency the ostium and recurrent sinusitis seems ble

relationship between the bacteriology in the l content and the pO_2 of the sinus was d in patients with acute sinusitis In a few of aerobic bacteria, the antral pO_2 was to zero while no cases with anaerobic ria had such low values However, a very antral pO_2 (3 mmHg) was also found in a without any bacteria in the sinus nor any in the antral washing

he low pO_2 in sinuses with small ostia or rbed ostial function can perhaps be a or in the development of sinusitis It has shown in animal experiments that if nitro was substituted for air in occluded tracheal ents, the transportation of mucus by the in the tracheal mucosa was decreased (Flot t al., 1960) The effect was reversible and when was introduced again the mucus transporta improved Possibly a low pO_2 in a paranasal s can have the same effect on the cilia of the al mucosa and be a factor in the develop t of sinusitis

acuum sinus has been discussed in connec with a low manometric pressure in the s (Sluder, 1927, Söderberg, 1934, Ballenger & enger, 1952) and with low oxygen and carbon dioxide content in the sinus (Flottes l, 1960) The low pressure is thought to e pain In our investigation there were 7 ents with sinusitis combined with pain in paranasal region Six of these had reduced ral oxygen tension in the sinus and only one the patients had a purulent discharge on ral washing A correlation between low gen content and pain in the sinus may thus possible but it is not the only explanation manometric pressure could not be measured hese patients for technical reasons

ZUSAMMENFASSUNG

pO_2 in der Kieferhohle gemessen mit einer pO_2 trode ist, wie grundlegend festgestellt wurde, von der Öffnung des maxillaren Ostiums und auch von der funktionellen Grosse des maxillaren Ostiums abhangig, wenn dieses einen kleineren Durchmesser als 2 5 mm hat In normalen Fallen wurden in der Kieferhohle eine durch schnittliche Sauerstoffspannung von 116 mmHg und bei Patienten mit Sinusitis ein durchschnittlicher pO_2 von 75 mmHg festgestellt Es wurde keine Korrelation zwischen dem Volumen und dem pO_2 der Kieferhohle festgestellt Der pO_2 bezog sich in Fallen mit Sinusitis nicht auf das Vorkommen und Nichtvorkommen von Eiter oder Schleim in der Kieferhohle In zwei Fallen mit aeroben Bakterien im Eiter der Kieferhohle wurde jedoch eine Sauerstoffspannung von nur wenigen mmHg gemessen Bei Schmerzen in der Gegend der Nebenhohlen wurde manchmal ein niedriger pO_2 festgestellt

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COMBINED RADIOLOGICAL AND SURGICAL THERAPY OF CANCER OF THE ETHMOID

Å Elner and Hj Koch

From the ENT Clinic, University Hospital Lund Sweden

(Received February 12, 1974)

Abstract A material of 12 cases of cancer of the ethmoid is presented The treatment was combined radiology and surgery with pre- and eventually postoperative radiotherapy and lateral rhinotomy with resection of the anterior base of the skull and dura mater in cooperation with a neurosurgeon Craniotomy of the conventional type was not performed In spite of the limited number of cases the results are considered promising and encourage treatment along the lines described in the article 9 out of 12 patients are free of recurrence with an observation time of 2-12 years Predisposing factors are discussed

Cancer of the ethmoid is a fairly uncommon disease According to "Cancer incidence in Sweden" (1968) there are about 70 cancers annually of the nose and paranasal sinuses or about 0 9/100 000 inhabitants Among these, of the maxilla is in the majority and pure ...moidal tumours form a minor part According to Macbeth (1965) cancer of the ethmoid constitutes about 1/3 of all cancers of the nose and paranasal sinuses, and according to Osborn & Winston (1961) about 30 %. Frazell & Lewis (1963) report a lower incidence, approximately 10 %, and Larsson & Mårtensson (1954) found somewhat less than 5 % cancer of the ethmoid among 379 cases of cancer of the paranasal sinuses

In Sweden the above mentioned figures would mean that the frequency of cancer of the ethmoid would range between 7 and 23 cases every year It is thus a limited group of tumours and in a small country like Sweden centralization of the treatment is necessary in order to achieve results that are on a level with those presented in the literature and are based upon a ... of cases

MATERIAL

We have operated on 12 cases of cancer of ... ethmoid with lateral rhinotomy and resec... the anterior skull base after preoperative ... therapy (Table I) There were 10 males ... females and the mean age was 50 years ... mean age in large materials of cancer of ... paranasal sinuses is usually somewhat ... but this material is small and a few ... patients considerably affect the mean age ... youngest patient was only 17 years ... seems, however, that the mean age in ... of cancer of the ethmoid is somewhat low... in a composite material of cancer of the ... nasal sinuses Because of its smallness, ... ratio in this material is impossible to ass...

PREDISPOSING FACTORS

A large number of factors predisposing ... of the ethmoid have been discussed (Ta... For a long time chronic infections of the ... and paranasal sinuses have been conside...

Table I *Cancer of the ethmoid operated ... rhinotomy and resection of the skull ba...*

No	Sex	Age
12	10 ♂ 2 ♀	17-68 Mean 50

: II *Predisposing factors for cancer of the oid*

nic sinusitis and tis	Öhngren 1933, Larsson–Mårtensson, 1954 Frazell–Lewis, *1963*, *Lewis–Castro, 1972*
sure to el dust	Doll 1958
sure to dust hard wood	Acheson–Hadfield–Macbeth 1967, Debois, 1969, Gignoux Bernard 1969, Hadfield–Macbeth 1971
ure to un wn factors in and leather stry	Acheson–Cowdell Jolles, 1970
tional intake itnt and nitrat	Druckrey et al, *1961*, *1962* Fong Walsh 1971

iore *common among cancer cases than* in a al population (Öhngren, 1933, Larsson & tensson, 1954, Lewis & Castro, 1972) Ined morbidity in cancer of the ethmoid is to be found among workers in nickel stry (Doll, 1958) A higher frequency has been shown among workers in the footwear leather industry (Acheson et al, 1970) It further been suggested that nitrosamines nitrite and nitrate in the food might be a ributory factor (Druckrey et al, 1961, 1962, g & Walsh, 1971) *There is statistical proof* a higher morbidity of cancer of the ethmoid, ve all adenocarcinoma, exists among workers he furniture industry in Buckinghamshire in land (Acheson et al, 1967, Hadfield & beth 1971) The observation that dust from d wood has somehow a carcinogenic effect also been confirmed by reports from Holland bois, 1969) and France (Gignoux & Bernard, 9)

mong 10 men in our material no less than re carpenters, *working in the furniture* istry Thus can hardly be a mere coincidence the number is higher than that statistically cted Also in Sweden there seems thus to be tiological *factor* connected with the working refinement of hard wood

PATHOLOGY

In unassorted material of cancer of the paraal sinuses there is a predominance of squamous cell carcinoma of different differentiation Adenocarcinoma is found in a minority of cases, and also papillary cylinder cell carcinoma or Ringertz' tumours There seems, however, *to be a certain difference in the distribution of* histological types according to the origin of the tumour Thus there are very few adenocarcinomas among the tumours of the maxillary antrum, where squamous cell carcinomas strongly predominate The normal ciliated respiratory epithelium undergoes metaplasia to squamous epithelium, from which the cancer originates Among cancers of the ethmoid there is no such predominance of squamous cell carcinoma and adenocarcinomas are more common Thus according to Frazell & Lewis *(1963)* there were *11* adenocarcinomas among 40 cancers of the ethmoid and Lewis & Castro (1972) found 16 adenocarcinomas and 47 squamous cell carcinomas among 63 cases of cancer of the ethmoid In a large material of nearly 400 cases of cancer of the nose and paranasal sinuses published in 1954 by Larsson & Mårtensson, the majority of the ethmoidal tumours were adenocarcinomas Out of 19 cases there were 11 adenocarcinomas

In our material adenocarcinomas constitute *50%* or 6 cases out of 12 *(Table III) Only* one case has been classified as squamous cell carcinoma and 3 cases are low differentiated or anaplastic tumours and 2 cases papillary cylinder cell carcinomas The anaplastic tumours were found among the youngest patients, 17, 27 and 37 years old It is a moot point whether the papillary cylinder cell carcinomas constitute a specific histological group of tumours or should be assigned to either squamous carcinoma or adenocarcinoma, depending on the direction in

Table III *Pathology*

Type	Number
Adenocarcinoma	6
Low differentiated or anaplastic cancer	3
Papillary carcinoma (Ringertz tumour)	2
Squamous cell carcinoma	1
Total	12

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patients considerably affect the mean [illegible]
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of cancer of the ethmoid is somewhat l[illegible]
in a composite material of cancer of t[illegible]
nasal sinuses Because of its smallnes[illegible]
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PREDISPOSING FACTORS

A large number of factors predispos[illegible]
of the ethmoid have been discussed (T[illegible]
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and paranasal sinuses have been cons[illegible]

Table I *Cancer of the ethmoid oper[illegible]*
rhinotomy and resection of the skull base[illegible]

No	Sex	Age
12	10 ♂ 2 ♀	17-68 M - 50

VI *Postoperative complications*

of complication	Number
ive bleeding	1
abscess	1 (†)
sy	1
	3

perative week Antibiotics were admin- d prophylactically for about 10 days after peration, mostly as a combination of peni- streptomycin (in early cases in this series) sulfa and in a few single cases chloramfeni

e postoperative complications were few le VI) In one case there was profuse arterial ling so that we had to ligate the external ad One case developed a brain abscess four s after the operation and died of this com- tion One case developed epilepsy which, ver, could be controlled without difficulty antiepileptic drugs The case with the brain ss was the only postoperative death

cause of local recurrence or progression of ual tumour—the latter expression ought to ie correct one when we are talking of ma int tumours—three cases were reoperated Table VII) In these cases we performed al rhinotomy and further resection of the of the skull and the dura mater

METASTASES

laterals of cancer of the nose and paranasal ses metastases to the regional lymph nodes he neck are reported in about 15–30% sson & Mårtensson, 1954, Macbeth, 1965, is & Castro, 1972) It seems, however, that astases are not quite as frequent in cases of cer of the ethmoid as in those of the antrum

le VII *Reoperation for local recurrence*

of operation	Number	Alive
otomy with further resection he skull base and the dura	3	2

Table VIII *Metastasis*

Twelve cases of cancer of the ethmoid	
Cervical lymph nodes	1
Distant metastasis	—

(Larsson & Mårtensson, 1954) Out of our 12 cases only one developed metastases in the regional lymph nodes in the neck (Table VIII) In one other case neck dissection was performed on a strong clinical suspicion of metastases, but this could not be verified at the histological examination Prophylactic neck dissection was not performed

RESULTS

Of 12 cases of cancer reviewed above 8 are alive without signs of tumour Two have died of their disease One died postoperatively and one of intercurrent disease 2 years after the operation (Table IX) At autopsy the patient who died of a brain abscess 4 weeks after the operation as well as the one who died of intercurrent disease after 2 years were found to be free of cancer and without metastases in the regional lymph nodes or in more distant locations If the patient who died postoperatively is considered dead of cancer, 9 cases are still alive The survival time varies but 7 out of these 9 cases have been alive for 6 12 years (Table X)

DISCUSSION

Cancer of the ethmoid is a fairly unusual tumour, at least in its pure form In the individual case with progression of the tumour to several sinuses it is always difficult to decide whether the origin was the ethmoidal cells or whether the tumour started in the maxillary antrum and then invaded

Table IX *Results*

Alive without signs of tumour	8
Died of cancer	2
Died postoperatively	1
Died of intercurrent disease	1
Total	12

Table X *Observation time in cases alive and without signs of recurrence*

Number of patients	Years
2	12
1	11
2	7
2	6
1	2
1	1 5

the ethmoid area In the literature, opinions differ as to the area where the tumour arises and this uncertainty is reflected in widely varying frequency figures According to some authors pure ethmoidal cancer is extremely rare, but according to others cancer usually starts in the ethmoid and especially in the so called antro ethmoidal cells, invading the maxillary antrum later (Macbeth, 1965)

As regards predisposing factors there seems to be statistical evidence that morbidity in cancer of the ethmoid is higher among people exposed to certain industrial air pollution but the specific carcinogenic substances have not been found or chemically isolated In addition to chemically active carcinogenic substances it is possible that unspecific irritation by air pollution may play a rtain role Snuff-taking has also been men- ned as an instrumental factor, but this is ubtful and a higher morbidity among people using snuff has not actually been demonstrated *Nor is there any certain connection between* tobacco smoking and cancer of the ethmoid (Hadfield & Macbeth, 1971) As we know that *the main part of the air inhaled through the nose* passes through the middle and upper nasal areas *it is reasonable to assume that the local carcinogenic substances* will act mainly in the ethmoid area There are no reports indicating that exposure to air pollution contributes to induce cancer of the nose and paranasal sinuses elsewhere *than in the ethmoid cells It has been* discussed, however, if not recurrent or chronic infections in the nose and sinuses may be of a certain importance for the origin of all forms of cancer of the nose and paranasal [illegible] also nasal polyps and squamous cell [illegible] are said to be significantly h[illegible] [illegible] hold this view have shown that up [illegible] cases *have been surgically trea*[illegible] or allergic diseases within nose [illegible] polyposis (Lewis & Castro, 1972) [illegible] than the statistical mean in a normal [illegible] Harrison (1973), however, [illegible] *this correlation With the* [illegible] *morbidity in certain areas owing to* [illegible] *substances in industrial work* [illegible] for example), cancer of the [illegible] exist in about the same frequency [illegible]

The ratio men-women [illegible] reports Larsson & Mårtensson [illegible] find any clear sex dominance [illegible] Lewis & Castro (1972) there is a [illegible] of men in the relation 2 to 1 [illegible] ethmoid cancer seems, however [illegible] that of cancer of the antrum so that [illegible] nate more strongly This is perhaps [illegible] in view of the different carcinogenic [illegible] that have been found in different [illegible] spite of the Women s Liberation [illegible] still predominate in the industries [illegible] which is likely to affect the sex ratio

As to the treatment, aggressive [illegible] therapy combined with radiotherapy [illegible] best possibilities of curing the [illegible] the tumour has invaded the anterior [illegible] skull and the dura mater, good [illegible] be achieved by resection of the areas [illegible] *A combined approach with fron*[illegible] and rhinotomy or the Dencker [illegible] been described by among others [illegible] *Kleitsch (1967)*, Ketcham et al. [illegible] Bridger & Shaheen (1968) We have [illegible] *these excessive operations* necessary [illegible] tion of the anterior base of the skull [illegible] mater can be done without [illegible] rhinotomy In our opinion coopera[illegible] neurosurgeon is of the greatest [illegible] *this situation*, since the otosurgeon [illegible] enter the skull cavity with the same [illegible] as a surgeon *trained in* neurosurg[illegible] that such team work provides [illegible]

-s to cure the patient and leads to better ts as a whole

stoperative complications have been re- ably few in spite of the fact that the area of y must be considered highly contaminated lthough meningus and brain have been laid This is probably due to the heavy anti- therapy administered as a routine In the ntibiotic era meningitis should probably been a much more common complication experience of the resorbable material used ry good It quickly induces fibrosis which tively prevents cerebrospinal fluid fistulas new "base of the skull' made up of con ve tissue was also found to be very strong e three cases reoperated on with further resection because of recurrent or residual our It is also somewhat surprising that only case developed postoperative epilepsy, cularly since in some patients the dura had resected and the brain surface covered with a and resorbable material or by resorbable rial only The absence of this complication obably due to the fact that no brain sub- ce was removed and that there was thus no for scars of the epileptogenic type

is well known in cancer surgery that chances ccess are limited in the case of reoperation ecurrent or residual tumour The patient's ce lies in the primary operation which must ade radical In our material there are, how- , three cases reoperated on for local re- ence, and two of these are still alive with a y long observation time without signs of l tumour or metastases This we think est that the prognosis of ethmoid cancer is ewhat better than that of cancer of the llary antrum, mainly owing to its tendency to produce metastases but to recur locally in ay which admits of successful surgical treat- t It seems likely that especially adeno- nomas of the ethmoid area develop neck astasis less frequently than tumours of the llary antrum (Larsson & Mårtensson, 1954) s also possible that the better prognosis of cer of the ethmoid is due to its tendency to symptoms early in contrast to cancer of the maxillary antrum, where symptoms often appear only when the bony walls have been destroyed by the tumour (Harrison, 1973)

According to our experience tumours invading bone can be favourably affected by radiotherapy if the tumour is radiosensible, but only in exceptional cases can all the tumour cells be killed and the patient cured As we know that diminished vascular supply and lowered oxygen tension in the tissues decrease the sensitivity of the tumour to radiotherapy it is also reasonable to provide the main part of the treatment preoperatively

It might seem irrational to provide preoperative radiotherapy in all cases of cancer of the ethmoid without regard to the histological type Adenocarcinoma is as a rule less sensible to radiotherapy Judging from the histological type it is however impossible in the individual case to decide if the tumour is going to respond to treatment or not Many adenocarcinomas react very well to radiotherapy and some of our best results were obtained in cases of adenocarcinoma We therefore find it justified to give preoperative radiotherapy as a matter of routine without regard to the histological type

In no case has the eye been sacrificed The capsula tenoni seems to be very resistant to tumours and only in one case did we have good reason, judging from the growth of the tumour, to exenterate the orbit In this particular case the patient did not allow us to remove the eye because he was blind on the other side Yet the rule must be that if there is the slightest sign or suspicion of tumour invading the orbit, this should be exenterated Some surgeons claim that the eye should be removed as a matter of routine because it is injured by the radiation and of little use to the patient Since, however, modern technique in radiotherapy allows effective shielding of the eye it is unnecessary to exenterate the orbit merely because of the risk of radiation damage to the eye

This material is of course too small to allow any certain conclusions as regards prognosis with the combined therapy described above We think, however, that the results tend to show

better figures than the overall figures for cancer of the nose and paranasal sinuses, and that thus further use of this combined radiological-otological-neurosurgical technique is indicated

ZUSAMMENFASSUNG

Eine Patientengruppe mit 12 Fallen von Karcinom des Ethmoidalsinus wird beschrieben Die Behandlung war eine Kombination von Radiotherapie und Chirurgie mit pre- oder eventuell postoperativer Strahlenbehandlung und lateraler Rhinotomie mit Resektion der vorderen [illegible]

von zwolf Fällen leben ohne Rezidive mit einer Observationszeit von 2 bis 12 Jahren Predisponierende Faktoren werden diskutiert

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HISTOCHEMISTRY OF PRIMATE LARYNGEAL MUSCLES

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·r The intrinsic laryngeal muscles of adult anes- d rhesus monkeys were removed by microsurgical que and studied for the following histochemical ns ATPase with and without acid preincubation · NADH, LDH SDH and PAS In the abductor ·, posterior cricoarytenoid, 60 % of the fibers had acid labile ATPase activity and 40 % of the fibers acid resistant ATPase activity, these muscle fibers xhibited a high content of oxidative enzymes. The muscle of the vocal folds, cricothyroid, showed a minance of acid labile fibers (70 %) and gave a mly strong reaction for oxidative enzymes. The arytenoid muscle contained 81 % acid labile and acid-resistant fibers The lateral cricoarytenoid and rytenoid muscles were almost exclusively composed d-labile fibers and showed a typical checkerboard n of oxidative enzymes.

laryngeal muscles are functionally very fast t, 1969, Mårtensson & Skoglund, 1964) ı & Cortesina (1965) demonstrated that like ateral rectus muscle of the orbit, the laryn- muscles showed the presence of up to 70)% of muscle fibers with multi motor end 's The percentage distribution of multi or end plate fibers in a muscle bore a direct ionship to its contraction time

ıe ultrastructure and enzyme contents of ngeal muscles have been reported by several ters (Berendes & Vogel, 1960, Ganz, 1971) 968, Hall Craggs, for the first time, reported ies correlating the contraction times and chemical picture of laryngeal muscles of rabbit. Syrovy & Gutmann (1971), employing biochemical techniques, found a direct relationship between the quantity of myosin ATPase and the speed of contraction of the thyroarytenoid and cricothyroid muscles of the rabbit

In this report we present the histochemical characteristics of the intrinsic laryngeal muscles of the rhesus monkey (*macaca mulatta*) thyroarytenoid, cricothyroid, posterior cricoarytenoid, lateral cricoarytenoid and interarytenoid An analysis of the histochemical profile of these very fast muscles is essential to an understanding of the biochemical nature of their motor units The findings of this study indicate that the majority of motor units of the intrinsic laryngeal muscles are composed of a unique fiber type that is both fast-contracting and fatigue resistant.

study was supported in part by U S Public Health ce Grant NS-08728 and SRS Grant 16-P 56809 5-05

MATERIAL AND METHODS

Intrinsic laryngeal muscles of adult rhesus monkeys (*macaca mulatta*), anesthetized with pentobarbital sodium, 25 mg kg, were removed by microsurgical technique The muscle tissue obtained was quickly frozen in isopentane and liquid nitrogen Serial, but not contiguous, 10–12 μm sections cut in a cryostat were studied for the following histochemical reactions

ATPase Myofibrillary ATPase activity was determined by the method of Padykula & Herman (1955) at pH 9 4 In this system, two main fiber types were distinguished light staining, low, ATPase activity and dark staining, high ATPase

better figures than the overall figures for cancer of the nose and paranasal sinuses, and that thus further use of this combined radiological-otological-neurosurgical technique is indicated

ZUSAMMENFASSUNG

Eine Patientengruppe mit 12 Fällen von Karcinom des Ethmoidalsinus wird beschrieben Die Behandlung war eine Kombination von Radiotherapie und Chirurgie mit pre- oder eventuell postoperativer Strahlenbehandlung und lateraler Rhinotomie mit Resektion der vorderen Schädelbasis und der Dura Mater in Zusammenarbeit mit einem Neurochirurgen Eine konventionelle Craniotomie wurde nicht durchgeführt Trotz der kleinen Anzahl von Fällen ist das Resultat als versprechend anzusehen Neun von zwölf Fällen leben ohne Rezidive mit einer Observationszeit von 2 bis 12 Jahren Predisponierende Faktoren werden diskutiert

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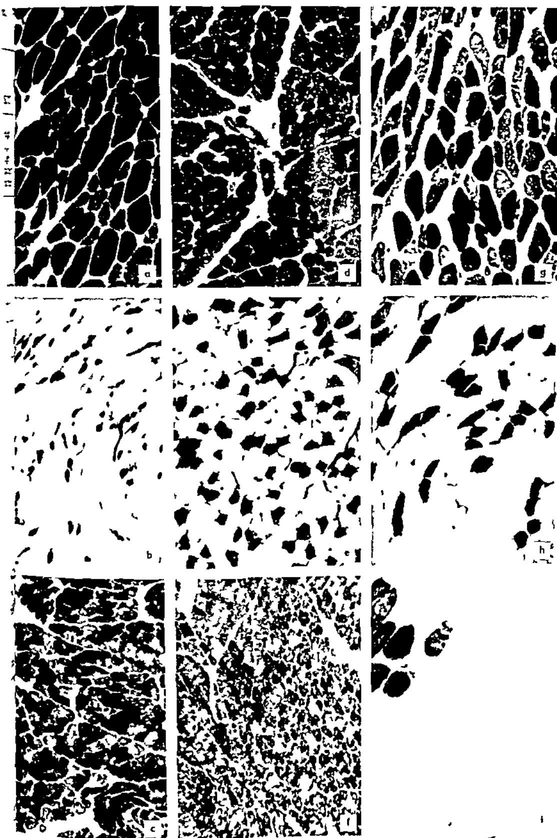

a
d
g
b
e
h
c
f

Table II *Comparison of acid-labile and acid resistant fibers (by ATPase* [illegible] *laryngeal muscles*

Muscle	Contraction time	% Acid resistant (%)	% Acid labile (%)	Acid resistant / Acid labile
Vastus medialis	50 msec (human)[a]	38	62	0 61
Thyroarytenoid	14 msec (primate)[b]	19	81	0 23
Cricothyroid	36 msec (primate)[b]	30	70	0 43
Posterior cricoarytenoid	44 msec (dog)	40	60	0 67
Lateral cricoarytenoid	19 msec (dog)	12	88	0 14

[a] Brooke & Engel (1969)
[b] Hast (1969)
Previous research has shown that the speed of contraction of canine laryngeal muscle is similar to the [illegible] monkey See Hast M H 1967 *Ann Otol 76* 489 Hast, M H & Golbus S 1971 *Pract* [illegible]

Acid preincubation results in a loss of myofibrillary ATPase activity in about 60 % of the muscle fibers The ratio of acid resistant to acid labile fibers was 0 66 (Fig 1*g*, *h*)

Lateral cricoarytenoid muscle in this muscle, a very large majority of muscle fibers showed strong actomyosin ATPase reaction Acid preincubation however, resulted in a loss of myofibrillary activity in the majority of muscle fibers The ratio of acid resistant to acid labile fibers was 0 14

Interarytenoid muscle in this muscle, a large majority of the muscle fibers also showed high actomyosin ATPase activity With acid preincubation the majority of muscle fibers lost their myofibrillary ATPase activity

DISCUSSION

The speed of muscle contraction has been directly correlated with the concentration and pH characteristics of actomyosin ATPase activity (Barany, 1967 Guth & Samaha, 1969, Brooke & Engel, 1969) In the gastrocnemius muscle of the cat, fast twitch motor units (contraction time 16–30 msec) have been shown to have high ATPase activity which is acid labile and alkali-stable (Burke, 1967, Yellin & Guth 1970, Burke et al , 1971)

Among the laryngeal muscles the thyroarytenoid and lateral cricoarytenoid are the fastest (contraction times 14 and 19 msec) [illegible] high ATPase activity and a [illegible] fibers with acid labile myosin ATPase [illegible] The corresponding oxidative [illegible] however, exhibit a more or less [illegible] indicating that these muscle fibers [illegible] oxidative metabolism

The cricothyroid (vocal fold [illegible] posterior crico-arytenoid (the [illegible] muscles have contraction times of [illegible] msec, respectively Their ATPase [illegible] a mixture of dark and light reacting [illegible] the dark fibers being acid labile [illegible] has been observed in the human [illegible] The oxidative enzyme pattern in the [illegible] shows a mixture of intermediate and [illegible] ting fibers, while in the posterior [illegible] the pattern is more homogeneous [illegible] tion is reminiscent of the mixed [illegible]

In conclusion, the faster [illegible] muscles are rich in ATPase [illegible] slower contracting muscles show a [illegible] distribution The ratio of acid [illegible] resistant ATPase activity is d[illegible] the speed of contraction The [illegible] distribution pattern shows that all the [illegible] muscles have high activity [illegible] been observed in the extraocular [illegible] 1969), human intrafusal muscle [illegible] et al , 1972) and in the tensor [illegible] pedius muscles of the cat ([illegible]

ZUSAMMENFASSUNG

_nneren laryngealen Muskeln von erwachsenen
_esierten Rhesusaffen wurden durch eine mikro-
_nsche Technik entfernt und folgende histochemi-
_eaktionen dann untersucht ATPase mit und ohne
_orbehandlung bei pH 4 4, NADH, LDH, SDH
AS In dem Abduktor, musculus cricoarytenoideus
_ior, hatten 60 % der Fasern eine hohe säure labile
_se Aktivitat, 40 % der Fasern zeigten eine saure
_nte ATPase-Aktivitat, diese Muskelfasern zeigten
_lls einen hohen Gehalt an oxydativen Enzymen
_ensor der Stimmbander (musculus cricothyroideus)
_eine uberwiegende Menge von saure labilen Fasern
_ und gab eine gleichmassig starke Reaktion fur
_tive Enzyme Der thyroarytanoide Muskel bestand
_% saure labilen und 19 % saure resistenten Fasern
_ateralen krikoarytanoiden und interarytänoiden
_eln waren fast ausschliesslich aus Fasern mit labilen
_se zusammengesetzt und besassen ein typisches
_brettmuster von oxydativen Enzymen

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ENZYME HISTOCHEMISTRY OF SQUAMOUS CELL CARCINOMA OF THE LARYNX

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Abstract Based on the clinical observation that the prognosis of larynx carcinoma is much more favorable in the female than in the male, biopsy specimens from 36 untreated patients (32 males and 4 females) were studied in an attempt to determine whether this apparently sex linked divergence in tumour behaviour is also reflected in the enzymic histochemical pattern characterizing these tumours From this material it is apparent that the enzyme pattern characteristic of squamous cell carcinoma of the larynx closely resembles that of analogous tumours with other localizations In addition the activities of various enzymes—although showing a great deal of individual variation—correlate with the degree of morphologic differentiation of the tumour Finally it is concluded from the relatively scanty material at our disposal that there are neither clear-cut nor consistent differences in the histochemical pattern of larynx carcinoma according to sex of the host

It is a well-established clinical fact that the prognosis of laryngeal carcinoma is much more favorable in the female than in the male (Kirchner & Malin 1953 Piquet, 1958) The present study was performed in an attempt to determine whether this apparently sex linked divergence in tumour behaviour is also reflected in the enzymic histochemical pattern characterizing these tumours

MATERIAL AND METHODS

The material consisted of biopsy specimens from 36 untreated patients (32 males and 4 females) which contained adequate amounts of neoplastic tissue without a degree of infection or necrosis that could possibly interfere with the correct interpretation of the enzyme co… neoplastic epithelial cells

The tumours were graded as h… moderately (Grade II) or poorly … ferentiated (Grade III) carcinoma … dence of tumours of various grades … Table I

Punch biopsy samples were quick… carbon dioxide, serially cut on a … microtome, and processed to dem… activity of the following enzymes … acid phosphatase, 5-nucleotidase al… phosphatase, non specific esterases (… used naphthol AS D acetate and … and α naphthyl acetate), leucylam… succinic dehydrogenase, lactic d… β-hydroxy-butyric dehydrogenase … hydrogenase, glucose 6-phospha… de… ase., α-glycerophosphate dehydro… uridine diphosphoglucose dehyd… techniques applied have been descr… (Thiery, 1963, Willighagen et al … the demonstration of succinic d… the slides were pretreated with PMS

Several frozen sections fixed in … dehyde were stained with hematox… periodic acid schiff (PAS, with dias… control), and Oil red O

RESULTS

The activity range of some of the … enzymes and the distribution of g…

I *Incidence of tumour grades in the present ... according to sex*

...ous cell ...ma of	*n* cases	
	Male	Female
I	18	2
II	11[a]	1
III	3 (5)	1
	32	4

[a] ...of these tumours also contained appreciable areas ...e III squamous cell carcinoma. Thus, the number ...lastic carcinomas of the larynx studied in the male ...s to 5, as indicated within parentheses.

neoplastic squamous cells are given in Tables II and III, for the male and female patients, respectively. Tumours were classified according to the grade of morphologic differentiation.

Squamous cell carcinoma in male patients

Larger quantities of glycogen are found more often in differentiated than in the more anaplastic tumours. As noted in squamous cell carcinoma in general, PAS-positive material tends to be distributed in the keratinizing neoplastic cells.

II *Range of activity of certain enzymes and distribution of glycogen in squamous cell carcinoma ... larynx in males*

	PAS	Af	ZI	5N	α-est
...r grade I					
	0 +	0	+	0	−
	0 + −	0	−	0	− − −
	0 + +	0	±	0	+
	0 − +	0 loc ±	− −	0 ±	− −
	0	0		0	±
	0 loc − +	0	±	0	+
	0 +	0	±		0
	0 loc − − +	0	±	0	− −
	0 − −	0	+/−	0	
	0 + +	0	±/+		+
	0 ±	0	±	0	− −
	0	0	+	0 loc ±	−
	0 loc −	0	±	0	− −
	0 −	0	0 +	0	− −
	0	0	+	0 +	
	0 −	0	+ −	0	−
	0	0	±	0	
	0 + +	0		0	
...r grade II					
	0	0	− −	0	− −
	0	0	+ +	0	/− −
	0	+ (+ +	+ +	0	+
	+ −	0 loc ±	+	±	± + −
	0 loc +	0	±	0	+
	0 loc +	0	+	0	− +
	0	0	− −	0	−
	0	0	+/+	0 loc −	+/+
	0	0	+	0 ±	−
	0	0 loc + +	+/−	0	− +
	0	0		0	+
...r grade III					
	0	−		0	0
	0 loc ±	0		0	−/+ −
	0	+		+	+
	0	0	+	0	
	0	0 ±	−	− +	0 loc +

[a]Tumours also containing areas of grade III squamous cell carcinoma. PAS = glycogen. Af = alkaline phosphatase; ...acid phosphatase. 5N = 5 nucleotidase. α-est = α naphthyl esterase. 0 = no enzyme activity. ± = trace activity. ... weak activity. + + = moderate activity. + + + = high activity. + + + + = very high activity. loc = local activity.

Table III *Activity range of certain enzymes and distribution of glycogen in squamous ... the larynx in females*

Tumour grade	Case no	PAS	AI	ZI	5N	α-est
I	33	0 +	0	+ +/+ + +	0	+/+ +
	34	0 loc –	0	+ +	0	+
II	35	0	0	+	0	+
III	36	0 loc –	0	+	+	/

Key see Table II

Larynx carcinoma is usually void of alkaline phosphatase activity, but with progressive dedifferentiation the squamous cells more often display some histochemically demonstrable activity of this enzyme The same trend is known to exist in carcinoma of both the uterine cervix (Thiery & Willighagen 1966) and the oesophagus (Willighagen unpubl)

In carcinoma of the larynx the activity of acidphosphatase varies considerably A definite relationship with the degree of morphologic differentiation is not conspicuous but in one of the present tumours (no 25) containing two clones of neoplastic cells acid phosphatase activity was clearly lower in the anaplastic tumour population

Although only weak (or even absent) activity of *5* nucleotidase is demonstrable in the neo...stic squamous cells The enzymic content ...these cells increases with dedifferentiation ...phenomenon also displayed by one of the tumours (no 25) which contained a double cell population

Tumour cells show divergent activities of non specific esterases which are most clearly shown with *α naphthyl acetate* as substrate and range from 0 to It is our contention that the mean activity correlates with tumour grade being highest in Grade I tumours This phenomenon is understandable because in squamous cell carcinoma of the uterine cervix (Thiery & Willighagen 1966) and the lung (Willighagen et al 1963) non specific esterase activity coincides with keratin formation

In contradistinction with the findings in cervical carcinoma (Thiery & Willighagen ...) the overall activity of lactic dehydrogenase ... squamous cell carcinoma of the larynx ... with differentiation This however ... illogical if it is taken into consideration ... basal and parabasal neoplastic cells ... show the most intensive staining, whereas ... pearls are almost devoid of demonstrable activity The activity of α glycerophosphate dehydrogenase on the other hand, ... seem to be correlated with the degree of morphologic differentiation In larynx carcinoma the relative intensity of the dehydrogenases studied is essentially similar to that found in malignant tumours in general (Willighagen ... 1963) and can be summarized as follows ... dehydrogenase > succinic dehydrogenase ... PMS) > α glycerophosphate dehydrogenase ... OH butyric dehydrogenase ... phate dehydrogenase undefined ... dehydrogenase

No cellular activity of adenosinetriphosphatase was found in larynx carcinoma except ... Grade II tumour (no 25) where the cells ... were clearly indicated by the stained ...

In some of the Grade I tumours ... ATP positive (Langerhans) cells were ... These elements are characteristic for ... *and pathologic squamous epithel*ium (... Willighagen 1969*a* and *b*)

Although no aminopeptidase ... present in the neoplastic epithelial ... tive tissue surrounding neoplasms ... invariably showed activity of this enzyme unrelated to the degree of tumour d...

IV *Average enzyme activity and distribu f glycogen and lipids in squamous cell car a of larynx according to tumour grade*

e	Tumour grade I	II	III
ne phosphatase	0	0	+
phosphatase		+	
leotidase	0	0	+
ninetri hosphatase	0	0	0
thyl esterase	+	(	+
laminopept dase	0	0	0
dehydrogenase		+	
trophosphate			
drogenase	+		
gen	0	0	0
	0	0	0

ls could not be demonstrated in neoplastic except in necrotic areas

mous cell carcinoma in women

to the rarity of laryngeal carcinoma in the l- the number of lesions studied is small does not permit the drawing of definite lus ons The available data strongly suggest -ver that roughly the same correlation be n tumour grade and enzyme activities and ibution of glycogen and lipids is found in inoma affecting men and women Further e a clear-cut d fference between the sexes respect to the histochemically demonstrable m- cortent of the tumour seems improbable

CONCLUSIONS

m th s selected series of biopsy specimens apparent that the enzyme pattern character of squamous cell carcinoma of the larynx elv resembles that of analogous tumours 1 other localizations for example the uterine ix (Th ery & Willighagen 1966) In addition activities of various enzymes—although wing a great deal of individual variation— relate with the degree of morphologic differ ation of the tumour This is clearly shown Tab. IV which gives a highly simplified presentation of the histochemical profile Finally it is concluded from the relative scanty material at our disposal that there are neither clear cut nor consistent differences in the histochemical pattern of laryngeal carcinoma according to sex of the host

SUMMARY

1 The activity of a number of hydrolytic en zymes and dehydrogenases and the distribution of glycogen and lipids were studied in 36 cases of laryngeal carcinoma (32 males and 4 females)

2 The enzyme histochemical pattern charac terizing this tumour closely resembles that of squamous cell carcinoma of other origin

3 A definite correlation between tumour grade and enzyme pattern was found to exist With higher degrees of differentiation tumour cells tend to show higher activities of alkaline phos phatase 5-nucleotidase and lactic de-hydro- genase whereas the reverse holds for non specific esterases The glycogen content is highest in the differentiated neoplastic squamous cells

4 The sex linked difference in biological be haviour characteristic of laryngeal carcinoma was not found to be clearly reflected in the histo- chemical pattern characterizing these tumours

ZUSAMMENFASSUNG

Aufgrund der klinischen Beobachtung, dass die Prognose des Kehlkopf-Carcinoms bei Frauen viel gunstiger ist als bei Mannern, untersuchten wir Biopsiematerial von 36 unbehandelten Patienten (32 mannlichen und 4 weiblichen) Wir versuchten, festzustellen, ob dieser offensichtliche geschlechtsgebundene Unterschied im Tumorverhalten sich auch im histochemischen Enzymmuster widerspiegelt, das diese Tumoren charakterisiert. Aus diesem Material ist ersichtlich, dass das fur das Plattenepithel-Carcinom des Kehlkopfs charakteristische Enzymmuster sehr grosse Ähnlichkeit mit dem analoger Tumoren anderer Lokalisation aufweist Ausserdem korrelieren die verschiedenen Enzymaktivitaten—trotz grosser individueller Schwankungen—mit dem Grad der morphologischen Differenzierung des Tumors Schliesslich wird aus dem relativ kleinen uns zur Verfugung stehenden Material geschlossen, dass weder scharf begrenzte noch einheitliche Unterschiede im histochemischen Enzymmuster des Kehlkopf-Carcinoms in bezug auf das Geschlecht des Carcinomtragers bestehen

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— 1969*a* The occurrence of the La[illegible] *Zool Path Antverp ensis 4*[illegible] 13[illegible]

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LUNG FUNCTION BEFORE AND AFTER LARYNGECTOMY

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act Five patients were examined with respect to onary mechanics before and after laryngectomy ods were used, the results of which were not in- ced by the laryngeal stenosis before and by any ss of the tracheostoma after the operation. It was n that the pulmonary resistance from trachea to a was normal before operation. Postoperatively onary resistance increased and reached a maximum six months, when it was about ten times as high as eratively One year after operation it was not more about twice the original value. At that time lung func was normal as concerns working capacity and arterial d gases A great adaptability of the airways was shown. The dangers and problems during the period ption are discussed.

ing the postoperative period laryngectomized ents often suffer from respiratory symptoms to the elimination of the air-conditioning ction of the upper airway The lung function ore and after laryngectomy has been studied others (Heyden, 1950, Fourage, 1964), but interpretation of the results has been com ated by the influence of the varying degree stenosis caused by the cancer and the trache- oma.

n order to avoid such influences we have ited our determinations to such measures as not affected by the upper airways We have, example, determined the resistance of the gs and airways from the pleura up to the chea In contrast to earlier investigations we ve followed the lung function with repeated asurements until one year after operation

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MATERIAL

Ten consecutive patients undergoing laryngectomy, September–December 1970, because of laryngeal cancer, were studied before and after operation Five of these patients could not be examined as planned, for the following reasons (1) postoperative death, (2) pulmonary metastasis (the only woman), (3) mental disease, (4) uraemia, and (5) local infection around the tracheostoma The patients who completed the investigations were 5 men, with a mean age of 64 years (range 55–75), all of them with a history of regular smoking for 15–50 years As to age and smoking habits they are typical patients with laryngeal cancer The oldest subject (no 1) had a history of pulmonary tuberculosis 9 years before operation Repeated bacterial controls had since then been negative Roentgenological and spirometric investigation showed severe permanent damage to the lungs The patient suffered from exercise dyspnoea and from angina pectoris since a myocardial infarction in 1965

One patient (no 4) suffered from chronic bronchitis He had for several years had a cough with expectoration but had never sought medical advice The remaining 3 subjects had no history of lung disease A chest X-ray was performed at each examination There were no roentgenological signs of parenchymal disease except for those observed in patient 1 No changes were observed during the postoperative period

Table I *Age and static lung volumes*

Total lung capacity, TLC, residual volume RV, vital capacity, VC, and functional residual capacity FRC are measured values in percent of values predicted according to Grimby and Söderholm (1963) w = weeks and m = months after operation

		TLC, % of predicted value					RV, % of predicted value			
Subject no	Age years	Pre op	2 w	2 m	6 m	12 m	Pre-op	2 w	2 m	6 m
1	75	103	79		100	97	183	117		151
2	63	86		87	108	97	84		116	108
3	62	104	86	89	102	106	117	113	104	115
4	65	109	97	107	113	114	125	129	167	131
5	55	125	101	110	116	107	145	127	132	135
Means	64	105	91	98	108	104	131	122	130	134

METHODS

Each patient was examined before, and 2 weeks, 2, 6, and 12 months after laryngectomy Pulmonary resistance, static elastic pressure volume curve and static lung volumes were determined Twelve months after laryngectomy a test was performed of the working capacity on a bicycle ergometer During this test arterial blood gases were examined as well as the resistive work rate of breathing, the functional pulmonary resistance and the breathing pattern

Pulmonary resistance and static elastic pressure volume curve

Pulmonary resistance and the static pulmonary pressure volume curve were measured with the flow regulator method (Jonson, 1969) This method allows determination of pulmonary resistance under standardized conditions with regard to breathing pattern and flow rate Resistance was in the present investigation measured at a flow rate of 1 l s throughout long expirations starting at lung volumes close to the total lung capacity Resistance was related to the static recoil pressure of the lungs Pst (1) This was done as Pst(1) is a very important determinator of resistance (Fry, 1958, Butler et al, 1960, Jonson, 1969, 1970*a*, *b*, Stubbs & Hyatt, 1972, Colebatch et al, 1973) When resistance determinations are made for the purpose of revealing pathological changes within the bronchi the values of resistance must consequ… tained at given values of Pst(1) (J… and *b*) In the present study values of … refer to expirations at 1 l s and a Pst(1) o… H_2O, if not otherwise stated

Only those details which differ fr… the original method (Jonson 1969) … into further consideration

The pressure difference between oes… and mouth includes the pressure d… extrathoracic airways It can therefore … used in the present investigation … fluence of these airways must be el… have measured the pressure difference … between oesophagus and mouth b… tween oesophagus and trachea in order … the pulmonary intrathoracic resistance … The pressure in the oesophagus was … via a balloon (Jonson, 1969) and th… trachea via a polyethylene catheter w… hole close to the closed tip The trach… was introduced under local anaesthe… direct laryngoscopy at the preopera… nation Postoperatively a tracheal ca… tex no FH36) was inserted and … avoid leakage The tracheal catheter … pressure recordings was passed d… the cannula so that its tip was … 2 cm above the carina The same p… catheter tip was aimed at pre and p… tively This was achieved by … position of the tip relative to the …

of predicted				FRC, % of predicted value				
2 w	2 m	6 m	12 m	Pre-op	2 w	2 m	6 m	12 m
62		81	67	124	87		129	126
63	83	113	96	83		78	142	100
67	78	82	88	125	94	92	125	122
84	76	96	96	131	106	158	139	145
87	100	104	92	129	129	140	151	143
73	84	95	88	118	104	117	137	127

subject the estimation was checked by and found to be adequate

pressure in oesophagus and trachea was ' by separate transducers (EMT 34, Elema AB) and the difference calcu- by an electronic subtraction unit

tic lung volumes Total lung capacity, TLC, volume, RV, vital capacity, VC, and onal residual capacity, FRC, were deter- with a body plethysmograph (Jonson,

ercise and lung function test One year after gectomy a graded exercise test on a bicycle was performed During this test arte- ood samples were drawn and analysed with (Eschweiler & Co) with respect to al pressure of oxygen, Pa_{O_2} and carbon di- Pa_{CO_2} Pulmonary functional resistance, and minute ventilation were measured at load Rf(l) is the viscous resistance of the including the extrathoracic airway at a taneous breathing pattern during exercise on & Jonson, 1973)

test an air-tight soft mask around the tracheostoma for the periods required for measurement of pul- ry mechanics The mask, which did not the tracheostoma, connected the latter pneumotachograph The flow signal from h and the signal represent- l pressure were fed to the com- (PDP-8, Digital Equipment) which per- formed the calculations (Jansson & Jonson, 1973) The load on the bicycle ergometer was step-wise increased until the patient was exhausted.

Subjects 1, 3, 4 and 5 were given 0 5 mg atropine subcutaneously 1 hour before each investigation This was done as the first patient (no 2) showed an increased rate of secretion which caused discomfort and technical problems during the measurements This subject was not given atropine even at the later investigations, in order to allow for comparisons of data All subjects were anaesthetized with a spray of 1–2 ml of Xylocain® (Lidocain) into larynx or trachea

RESULTS

The preoperative study showed a somewhat poor elastic recoil of the lungs at low volumes in all patients (Fig 1) FRC and RV were higher than expected TLC was normal and VC accordingly on the average slightly lower than expected (Table I)

The static elastic pressure volume diagram did not change after operation, nor did RV Two weeks and 2 months after operation FRC was slightly lower than before, but increased later to values just above those observed preoperatively TLC and accordingly VC decreased considerably after operation, but increased later to values close to those observed preoperatively

Resistance measured from pl to mouth (Rl) before operati

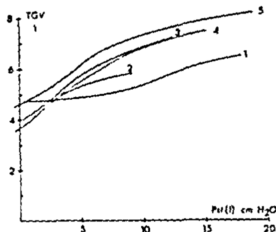


Fig 1 The elastic recoil pressure of the lungs was lower than in normal subjects (shadowed area), especially in the low volume range of each patient Pst(l) is recoil pressure, *TGV* is thoracic gas volume

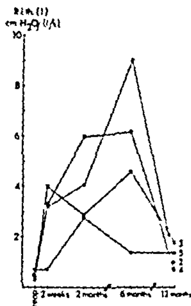


Fig 2 The resistance of the intrathoraci[c] Ri th(l), was normal before operation, ... reached a maximum about 6 months after ... turned towards normal values.

normal in subjects 1, 3 and 5 (Table II, normal range 1–3 cm H_2O/(l/s)) In the same subjects the intrathoracic resistance Ri th(l), was, on average, 5.4 cm H_2O/(l/s) lower (Table II) This difference corresponds to the resistance of the airways from the tracheal catheter tip to the mouthpiece It is much higher than normal values (roughly 1 cm H_2O/(l/s)) (Ferris et al, 1964) The high value can be attributed to the stenosis caused by the tumour Subjects 2 and 4 showed normal values of resistance in spite of the tumour According to the data of Ferris et al (1964) normal values of Ri th(l) can be estimated to range between 0.3 and 1 cm H_2O/(l s) All subjects except no 1 had normal values of Ri th(l) before operation In subject 1 the value is explained by his lung disease

After operation Ri th(l) increased in ... jects (Table II and Fig. 2) In ... Ri th(l) had increased more than ... months after operation After 12 months ... had fallen to values about twice as ... fore operation

In order to find out if the high values ... sistance observed 2–6 months after ... were due to bronchospasm, determ... Ri th(l) was repeated in 3 patients after ... nistration of 0.5 mg adrenalin subcut... In subjects 1 and 2 no change of Ri th ... observed In subject no 4 (6 months after ...

Table II *Resistance before and after operation*

R l is resistance including the extrathoracic airway before operation Ri th(l) is resistance from pleura to trache... Rf(l) is the functional resistance during exercise (Jansson & Jonson, 1973) including the resistance of the tra...

Subject no	R l cm H_2O (l s) Preop	Ri th(l) cm H_2O (l s) Preop	After operation 2 weeks	2 months	6 months	12 months	Rf(l) cm H_2O (l s) 12 months
1	13.1	5.0	15.3	8.6	19.5	7.0	5.3
2	1.4	0.3	3.2	4.1	9.1	1.0	1.1
3	4.6	0.4	4.0	2.9	4.6	1.8	1.9
4	1.5	0.5	3.3	6.0	6.2	0.7	1.1
5	6.6	0.7	0.7	2.8	1.4	1.4	7.4

III *Results from the exercise test 1 year laryngectomy*

redicted work load is based upon the age of the ts and unpublished normal material The maximum load is the highest sustained for 5 minutes The in parentheses refer to the work load at which the se test was interrupted, usually after 2 minutes gases were measured at rest in the recumbent n, and immediately before interruption of exercise

Max work load predicted	(kpm/min) performed	Pa_{O_2} (mmHg) At rest/ At work	Pa_{CO_2} (mmHg) At rest/ At work
600	200 (400)	65/80	38/36
600	600 (800)	not measured	
600	600 (600)	72/90	40/36
600	1 000 (1 000)	94/91	38/39
900	600 (600)[a]	78/92	41/42

r surgical revision of the stoma subj no 5 per- 1 900 kpm min for 6 minutes

Ri th(l) fell from 6 2 to 3 6 cm H_2O (l/s) ubject did not experience any sensation re- to this fall of resistance

jects 2–4 had normal or even high work- apacity one year after operation according r standards and those of Åstrand (1960) ct no 1 had preoperatively a low working ity which was further reduced postoper- y Subject no 5 demonstrated a moderate tion of his working capacity, well correlated dyspnoea This subject suffered from a ste- of the tracheostoma due to shrinking, developed 6–12 months after operation onsequence the functional resistance, mea- from pleura to atmosphere during the se test, was considerably increased and higher than Ri th(l) measured from pleura chea (Table II) After surgical revision of acheostoma the working capacity became al (900 kpm/min) The other subjects showed r values of Ri th(l) and Rf(l) They had usly small values of tracheal resistance

rest Pa_{O_2} was at about the lower limit of ormal range in 2 subjects, Pa_{CO_2} was nor- Table III) During exercise, blood gases at all loads in all patients

DISCUSSION

In 5 out of 10 patients the investigations could be completed These 5 patients were of an age and had a history of smoking corresponding to most subjects with laryngeal cancer

The pulmonary disease in one patient (no 1) may have influenced the results The course, however, was similar in the 5 patients Furthermore, the incomplete data from patients not completing the investigation show the same pattern In spite of the small number of patients the uniform results make us believe that the results in a representative way show how the lung function is affected by laryngectomy

Complications such tracheitis and bronchitis often follow the disconnection of the upper airways from the respiratory pathway (Ingelstedt, 1956, Toremalm 1960) These problems tend to diminish after a varying period of time Heyden (1950) could not demonstrate any change of lung function or of working capacity 1½–7 months after laryngectomy Torjussen (1968) reported that disturbed lung function after laryngectomy was often caused by stenosis due to shrinking of the tracheostoma Fourage (1964) showed that no major deterioration of spirometric data followed 1 2 months after laryngectomy Most of his patients had low values of forced expiratory and inspiratory volumes, FEV and FIV, respectively, in the preoperative study In several cases FIV improved considerably due to the elimination of the extrathoracic obstruction caused by the cancer The values of FEV were often unchanged In the light of our data this may be due to the development of an obstruction in the intrathoracic airways instead of the eliminated cancer Fourage (1964) showed that no significant changes of blood gases existed 1–2 months after laryngectomy

No previous study of pulmonary resistance or elastic properties after laryngectomy seems to have been done Nor have previous authors repeated the determinations on several occasions postoperatively The determination of the resistance of the lungs up to trachea has not only the advantage that obstructions due to the cancer

or tracheostoma are excluded It also increases the sensitivity of the method as concerns its ability to detect obstructions in the lower airways, as the resistance of the extrathoracic airway, *already in normal subjects high and variable*, is by passed, Ri th(I) was on average, 0 5 cm H_2O/(l s) preoperatively (subject no 2-5) Accordingly, no bronchial obstruction could be shown preoperatively in subjects 2-5, in spite *of their heavy smoking habits and as concerns* subject no 4 clinical signs of bronchitis It must be pointed out that the administration of atropine to 4 out of 5 subjects may have eliminated reversible bronchoconstriction

The poor recoil of the lung preoperatively may to some extent be explained by a higher age in our patients compared with our normal subjects (Fig 1) (Mead et al, 1967) It might also be due to an increased tendency to bronchial closure at low lung volumes a phenomenon which is a likely explanation of the high RV as well (Jonson, 1970*b*) The normal values of resistance should not be taken as a proof of healthy airways in our patients in the preoperative study We can, however, conclude that the obstruction of the extrathoracic airway due to the cancer observed in subjects 1, 3 and 5, had caused no major changes in the intrathoracic airways

The high values of Ri th(I) obtained postoperatively with the flow regulator method at a standardized value of elastic recoil pressure reflect specifically changes of the bronchial tree (Jonson 1970*a* and *b*, Colebatch et al, 1973) Because of the lengthy procedure of the investigation measurements could be only repeated at a few occasions after bronchodilation The sparse data show an inconsistent and incomplete effect of adrenalin on the obstruction which indicates that bronchospasm is not of major importance as a causative factor No patient suffered from crustal tracheo bronchitis The nature of the obstruction is not clear Griffith & Friedberg (1964) and Schwab (1955) have shown that changes in the trachea essentially in the form of metaplasia and chronic inflammation will follow laryngectomy How far such changes extend into the deeper airways is not known In our investigation the obstruction does not s to be situated in airways smaller than ... 1 mm in diameter, since airway closure and ... fore higher values of residual volume and ... *ther decrease of elastic recoil* pressure sh... be expected at changes in small airways (J... 1970*b*)

The most likely explanation for the ... lung resistance postoperatively is an obstr... *due to an inflammatory state in the larger b...* chi During the first months after laryngec... the patients had more or less pronounced s... toms such as tightness of the chest, expect... and cough Such symptoms appear in the p... material to be very well correlated in time... intensity to the changes of Ri th(I). The s... toms and signs are well compatible with ... chitis One patient (no 5) experienced ... ing dyspnoea during exercise and a ... working capacity after one year Our mea... ments showed that Ri th(I) was normal ... the functional resistance, including the ... tracheostoma, was high After surgical ... of the stoma the patient had a normal w... capacity and no dyspnoea

Reasonable causes for the developm... bronchial obstruction are elimination of th... conditioning of the inspired air and ... mechanisms for elimination of secretion

All patients were treated with an ar... nose (Toremalm, 1960) after the oper... After 2 to 3 weeks the use of tracheal ... and artificial nose was discontinued. The p... were then instructed to carry moist gauze ... presses over the tracheostoma and drip ... into the trachea All patients have ch... carry dry gauze compresses continuously ... of them have spontaneously constructed m... ical devices for the gauze, so as to keep ... front of the stoma and avoid its interf... with inspiration The patients experience ... those measures and a loose arrangement ... clothing around the stoma facilitated the b... ing We feel that there is a need for a c... cially available device giving effective a... ditioning permanently

The elimination of secretion depends

liar function and the mechanism of cough ciliar function is lost to the extent that ous metaplasia occurs (Schwab, 1955, th & Friedberg, 1964) The effectiveness of is considerably decreased as the function ttis is lost The patients in time acquire a ique to improve the effect of their cough inspire to a high lung volume and simulate inction of glottis, by occluding the stoma r example, a compress, prior to the cough by they reach the high flow rates necessary xpectoration This cough technique should ight early and if possibly trained preopera-

The prominent reduction of Ri th(1) ring during the period 6–12 months after tion shows a remarkable ability of the air to adapt to the poor air conditioning and i mechanism

pattern of breathing at rest is very variable asily disturbed by emotional factors and ore difficult to study It has been reported review see Fourage, 1964) that the breath attern appears uneconomical in laryngecto- patients during the first few months after tion We have no data which allow us to whether this was the case with our patients s quite obvious, however, that the patients arge problems to control voluntarily the atory manoeuvres during the tests These ems have certainly contributed to changes ed in the static lung volumes especially otal lung capacity 2 weeks to 2 months after tion The unchanged elastic recoil of the after laryngectomy, does not indicate any se of the parenchyma responsible for a de- of TLC Later the TLC returned to values ar to those observed preoperatively

C was first decreased as has also been re- d by Bernhardsgrutter et al (1955) but then ased to preoperative values A reasonable nation for this may be a disturbed nervous ol of the respiration during the first few hs after laryngectomy

e breathing pattern during exercise is less ted by emotional factors One year after gectomy the breathing pattern during exer- was similar to that observed in normal subjects, as concerns tidal volumes, frequencies, flow patterns and pleural pressures This means that the patients did not procedure pleural pressures above 2–6 cm H_2O at heavy exercise, which is about optimal (*Olafsson & Hyatt, 1969, Jonson, 1970, 1971*) The nervous control of the ventilatory pattern during exercise was adequate in our subjects one year after laryngectomy

In summary one year after laryngectomy the lung function is in general remarkably good as judged from arterial blood gases, static lung volumes, resistive and elastic properties, nervous control of ventilation and working capacity During the period of adaptation the patients suffer from pulmonary symptoms In subject no 1, who had reduced lung function preoperatively, the symptoms were severe In order to reduce the evident risk for such patients and the troubles for the patients without major lung disease it is desirable to improve pre and postoperative treatment

ZUSAMMENFASSUNG

Es wurden die mechanischen Eigenschaften der Lunge bei funf Patienten vor und nach einer Laryngektomie untersucht Die verwendete Methode gewahrleistete dass eine Larynxstenose vor oder eine Stenosierung des Tracheostomas nach der Operation die zu messenden Lungenparameter unbeeinflusst liessen Praoperativ wurde zwischen Trachea und Pleura ein normaler pulmonaler Widerstand gemessen Dieser nahm jedoch postoperativ zu um nach etwa sechs Monaten bei ca zehnfacher Steigerung sein Maximum zu erreichen Ein Jahr nach der Laryngektomie war der pulmonale Wider stand wieder auf das Zweifache seines Ausgangswertes zuruckgegangen Zu dieser Zeit lag in bezug auf Arbeits kapazitat und Blutgase eine normale Lungenfunktion vor Auf diese Weise konnte eine weitgehende Adapta tionsfahigkeit der Luftwege an die nach einer Laryngek tomie veranderten Ventilationsbedingungen dargestellt werden Gefahren und Probleme wahrend dieser Adapta tionsperiode werden diskutiert

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FUNCTION OF THE LOWER OESOPHAGEAL SPHINCTER IN A POPULATION SELECTED AT RANDOM

A Manometric, Radiological, and Questionnaire study

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t Using a manometric technique, incompetence lower oesophageal sphincter (LES) was found in of forty persons selected at random The women y predominated with respect to LES incompe- mean pressure difference between the LES and al part of the oesophagus at the end of expiration mmHg in the subjects with LES incompetence 5 mmHg in the others Six of those with LES tence were found to have hiatal hernia Dys- of the oesophagus was common in the subjects ES incompetence On X ray examination, one of

etence gave a history of symptoms suggesting The symptoms are graded on a scale The difference e between the competent and incompetent LES is statistically highly significant ($p < 0\,001$) The ms from organs other than the oesophagus in d by the vagus nerve described by subjects with ent and competent LES are compared The mechanism of the oesophagus sphincter is dis-

ons differ concerning the incidence of sliding hernia and reflux in a normal ation (Skinner & Booth, 1970, Venkata- n et al, 1972) and in persons with gastro- nal symptoms (Beck, 1954, Stein & Finkel 1960) This is undoubtedly explained by fferent methods of examination and diffe- nteria for the diagnosis of hiatal hernia eflux Many different methods of X-ray examination to demonstrate hiatal hernia and/ or gastro-oesophageal reflux have been recommended Sommer & Stevenson (1961) used an angulated table, the patient lying prone with the epigastrium pressed against the angle of the table Johnstone (1951) examined his patients in the standing position, bending forwards as if to touch the toes Turning over from the supine to the prone position is recommended by some as the best manoeuvre to reveal hernia (Flood et al, 1953) Different ways of increasing the intra-abdominal pressure have also been used Coughing, taking deep breaths, or performing the Val salva or Mueller manoeuvres have been recommended Inflating a rubber belt placed round the upper abdomen has been practiced by Marchand (1952) and Sandmark (1963*b*) Sandmark connected the rubber bag to an electric fan, and with the aid of an autotransformer he was able to maintain a constant pressure at various chosen levels Wolf (1960) and Stein & Finkelstein (1960) made the examination with the patient lying down and actually swallowing the contrast medium Others examine the patient after the contrast medium has been swallowed Most authors use heavy barium contrast media (spec grav 1 4–2 0) Sandmark (1963*b*) used light contrast medium (spec grav 1 0), with an improvement in diagnostic accuracy

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When radiology reveals hernia or massive gastro-oesophageal reflux in the recumbent position without extra abdominal compression, the diagnosis may be taken as settled Many radiological techniques involve some artificial means of uncontrolled increase in the extra abdominal pressure resulting in an unknown increase in the intragastric pressure Such an increase in the intragastric pressure may in turn give rise to reflux and to hernia in normal subjects The results of radiology carried out when applying extra abdominal compression to demonstrate gastro-oesophageal reflux and hiatal hernia must be assessed in relation to other non radiographic findings and to the history, if they are to be accepted as reliable in distinguishing between normal and pathological findings

Symptoms due to dysfunction of the oesophagus have been imperfectly interpreted owing to the lack of a manometric technique in the routine examination of the oesophagus There can be no doubt that many symptoms in patients with oesophageal dysfunction are wrongly attributed to other neighbouring organs also innervated by the vagus nerve Bennett & Atkinson (1966) suggested that angina pectoris may often be of oesophageal rather than of cardiac origin Hiebert & Belsey (1961) and Urschel & Paulson (1967) found an unexpectedly increased incidence of oesophageal dysfunction among sufferers from bronchitis obstructive bronchitis and laryngitis

The purpose of the present investigation was to study the incidence of pathological manometric findings in a series of subjects selected at random and to establish the value of X ray examination with controlled abdominal pressure in revealing hernia and reflux Further aims were to correlate the objective findings to the symptoms and to compare any histories suggesting disorders of neighbouring organs innervated by the vagus nerve in subjects with normal and abnormal LES function

MATERIAL

From the population of a Swedish town with 36 028 inhabitants five women and five men were selected at random from each of the age groups 15, 25, 75 Thus 70 pers[illegible] invited to take part in the investig[illegible] declining were asked a second time For[illegible] plied, 24 men and 16 women Their ag[illegible] bution is shown in Fig 1

METHOD

The investigation consisted of manomet[illegible] X ray studies and a questionnaire The[illegible] were statistically evaluated by Student's [illegible]

Questionnaire

The questionnaire contained seventy que[illegible] concerning symptoms such as heartbur[illegible] regurgitation lump in the throat [illegible] heart pain gall bladder and gastric illness [illegible] and weight number of deliveries tobacc[illegible] sumption dentures and current drug the[illegible]

Manometry

The manometric study was performed [illegible] water filled polyethylene catheters of 0[illegible] inner diameter connected to external p[illegible] transducers (sensitivity ± (0-300) mmH[illegible] ma-Schönander Sweden) Each cathet[illegible] one lateral hole of 2 mm diameter placed [illegible] intra luminal pressures were recorded ei[illegible] three catheters 1 cm apart or in three ca[illegible] 5 cm apart The manometers used were [illegible] 34 (Elema Schönander Sweden) The pr[illegible] were graphed on a multichannel direct [illegible] recorder (Elema Schönander Sweden) w[illegible] paper speed of 1 cm second The pH-ele[illegible] placed in the distal part of the oesoph[illegible] a stomach electrode (LKB Beckman I[illegible] ments AB Sweden) connected to a Zer[illegible] (LKB Beckman)

The respiration was manometrically re[illegible] from a small air filled balloon attached t[illegible] lower chest and connected to a pressure t[illegible] ducer

The intra luminal tonus pressures were re[illegible] ded simultaneously at three points 1 cm a[illegible] The tube assembly was withdrawn at 0[illegible]

from the stomach to the pharynx at inter- of at least two breathing cycles

e motility pressures of the oesophagus were ded simultaneously at three points 5 cm The catheters were withdrawn in 1 cm at intervals of up to 1 minute depending e duration of the peristaltic wave Oeso al motor function was studied during owing The subject first swallowed water hen swallowed dry, or vice versa

th tonus and motility determinations were ted once

the last feature of the manometric exami- 1 extra abdominal compression was applied eans of a fan attached to a 15 cm-wide belt round the upper abdomen, as de d by Sandmark (1963*b*) The pressure was in controlled steps from 0–100 mmHg ressure belt was insufflated while the cath ssembly was lying with one hole in the ch and two holes in the oesophagus 5 cm the distal one close to the cardia When flux occurred 100 cc of 0 1 N HCl was d into the stomach, and the extra ab- al pressure was increased once again as bed above

subjects were requested not to smoke or r 4 hours before the manometric and X ray nations The tube assembly and the pH de were passed through the nose after nasal anaesthesia, and the subjects were ned in the supine position

e the pressure at a certain point was reg with three different catheters and the as repeated once, six values for the intra al pressure were obtained from the same Each figure given in the results represents ean of the six recordings

examinations were performed on all 16 n except 2 (one was pregnant and one did me) Two men who were shown by mano to have a hernia or an extremely low re gradient between the LES and the hagus were also examined

radiological technique was as follows The subjects were examined while swallowing barium suspension (spec grav 1 4) in the supine, right anterior oblique position (RAO position) with and without extra abdominal pressure of 100 mmHg applied (Sandmark, 1963*a*), and in the standing position with no extra abdominal compression During normal breathing, serial films of the hiatal region were taken at one frame per second with a 70 mm camera Fluoroscopy was used

The subjects were also examined after having taken about 100 ml contrast medium in the supine RAO position with the foot end at the table elevated 15 degrees The subject was asked to breathe deeply and to perform a Valsalva manoeuvre, and the extra-abdominal pressure was raised in steps from 0–100 mmHg The hiatus region was examined by fluoroscopy and spot films were taken Finally, with extra abdominal pressure still applied, they were asked to turn from the supine to the prone position

The diagnosis of hernia was made on the basis of one of the following criteria

1 A complete Schatzki ring (Schatzki & Gary, 1953),
2 An incisura cardia above the hiatus,
3 Obvious signs of gastric mucosa above the hiatus in combination with widening of the hiatus

RESULTS

The following definitions are used and they all refer to the supine position

1 By *reflux* is meant either the decrease of pH or a pressure increase in the distal part of the oesophagus with an extra abdominal pressure of 100 mmHg or less applied
2 *LES incompetence* means reflux as defined above or no pressure gradient between LES and oesophagus
3 Oesophageal *dysmotility* is indicated by,
   (*a*) Propulsion of the motility wave on dry swallowing at a rate exceeding 6 6 cm per second in at least two tests,

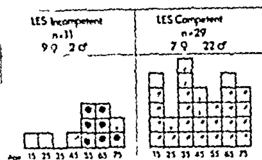


Fig 1 Age and sex distribution in the LES-competent and LES incompetent respectively ● = Hernias

(*b*) The following of the peristaltic wave by tertiary contractions on every occasion

Manometry

Eleven subjects had LES incompetence, all but one with demonstrable reflux (Fig 1) Six of these subjects were found to have a pressure curve indicating a hernia of less than 2.5 cm (Fig 2) Yet another subject was shown to have a hernia on X-ray examination on the application of an extra abdominal pressure of 100 mmHg

The subjects are arranged in two groups, LES-incompetent and LES-competent

In Fig 1 the age and sex distribution of the two groups is shown The LES incompetent group includes 9 women and 2 men, and hernias were present in 6 women and 1 man Since the Competent and Incompetent' groups differed with respect to age distribution, all the findings are assessed both in the whole series and in the part of the series related to subjects aged 55 years or more No sex difference can be found with respect to LES incompetence below 55 years of age However, from 55 years

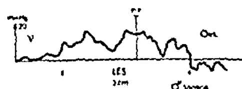


Fig 2 The tonus registration of the intraluminal pressure in one of the LES-competent subjects The plus and minus refer to the intraluminal pressure change during inspiration *V* = ventricle *PIP* = pressure inversion point *Oes* = oesophagus

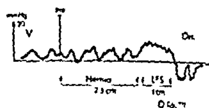


Fig 3 The tonus registration in one of the subje[illegible] a hiatal hernia For explanation of symbols see Fi[illegible]

and above there is a statistically sig[illegible] difference ($p<0.01$) in the incidence of LE[illegible] competence between the sexes No hern[illegible] found in any subject under 55 years of [illegible]

LES-competent subjects had a [illegible] of 7.1 mmHg in the LES at the end of [illegible]tion, and in the distal part of the oesop[illegible] of −3.4 mmHg, that is, a pressure grad[illegible] 10.5 mmHg (Fig 4, Table I) The LES-[illegible]petent group showed a mean LES pre[illegible] 1.8 mmHg and a mean oesophageal pres[illegible] −2.4 mmHg that is, a pressure gradient [illegible] mmHg (Fig 5, Table I) The difference be[illegible] the groups is highly significant ($p<0.0$[illegible]) [illegible]ing inspiration the intraluminal pressures [illegible] oesophagus and the LES changed proport[illegible] and inversely to each other The mea[illegible] gastric pressure increase was 10 mmH[illegible] extra abdominal pressure of 100 mmHg [illegible] I) The pressure increase did not differ be[illegible] the two groups

The mean length of the LES calcula[illegible] the pressure inversion point to the be[illegible] the part of the oesophagus showing sub[illegible]spheric pressure, and the mean length [illegible] upper oesophageal high pressure zo[illegible] slightly less in the LES-incompetent grou[illegible] in the LES-competent group The differe[illegible] not statistically significant

Questionnaire

The questionnaire concerning symptoms [illegible] ranked on a scale, each symptom being [illegible] according to the frequency with which it o[illegible] in the LES incompetent subjects (Tab[illegible] All but one in the LES-incompetent gro[illegible]quired more than 5 points (mean 8.5) The [illegible]competent subjects attained a mean score [illegible]

h is significantly lower than the other group 0 001, Table I) When comparing the symp- in the two groups of subjects, aged 55 years ore the difference was still statistically signif t ($p<0$ 01)

ible II shows the incidence of history of t pain, gall bladder and gastric disorder, and present smoking and dysmotility of

e I *The intraluminal pressure of the oeso-eal sphincters, ventricle and oesophagus, ht excess, length of the oesophageal sphinc and history score in LES competent and incompetent subjects*

results are given with standard error of the mean ster sks refer to significance limits of $p<0$ 05 (*) 01 (**) and $p<0$ 001 (***) The values within theses are those from the age groups 55 years or

	LES competent (*n* 29)	LES incompetent (*n* 11)
ure grad ent Hg) LES– phagus end of	10 9+1 15 (9 5±1 82)	4 2±0 83*** (3 6+1 07)**
ure gradient ventricle of exp	7 07+1 15 (6 2±1 53)	1 8+0 64*** (2 3±0 70)
ure gradient Hg) LES– ricle end of	13 1±0 89 (13 8+1 21)	−10 0±2.55 (11 1+3 34)
ure gradient Hg) oeso-gus-ventricle of exp	−3 4+0 64 (−3 4+1 01)	2 4±0 84 (−1 4+0 66)
ure grad ent Hg) oeso-gus-ventricle of insp	10 9+1 38 (12 4+0 96)	−9 8±1 09 (8 8±1 18)
ng intragastric sure (mmHg)	3 1±0 47 (3 3±0 54)	3 3+0 66 (3 9+0 77)
cially increased agastric pressure Hg)	9 3±1 03 (7 7+0 92)	10 7±2.36 (9 4±2 23)
h of LES (cm)	3 5+0 27 (3 1+0 23)	2 8±0 35 (2 5±0 37)
h of upper phagus high sure zone (cm)	3 52±0 27 (3 09±0 23)	2 8+0 35 (2 5+0 37)
ht excess (%)	7 6+2 87 (7 5+2 91)	10 9+3 48 (13 5+4 3)
tionnaire score	2 6+0 55 (3 3+0 99)	8 5±0 90*** (8 9±1 20)**

Table II *History of heart pain gall bladder and gastric disorders, smoking habits, full dentures, and occurrence of oesophagus dysmotility in sub jects with competent and incompetent LES*

Values in parentheses refer to subjects 55 years and over For explanation of asterisks see Table I

	LES incompetent	LES competent
Heart pain	7/11 64% (6 8 – 75%)	4 29 – 14% (3/12 = 25%)*
Gall bladder	2 11 18% (2 8 25%)	5/29 17% (3/12 – 25%)
Gastric disorder	3 11 27% (3 8 38%)	5 29 17% (1 12 = 8%)
Smoker	4 11 36% (1 8 13%)	10 29 35% (0 8)
Ex smoker	1 11 9%	8 29 28%
Full dentures	4 11 36% (4 8 = 50%)	4 29 14% (2 12 17%)
Dysmotility of oesophagus	6/11 – 55% (5 8 63%)	2/29 = 7% (0 12) **

the oesophagus In evaluating the variations in the different parameters it must be taken into consideration that most of them are age depend ent several are sex dependent, and some are interdependent Since there are very few subjects below 50 years of age in the LES incompetent group, statistical assessment is only carried out on the subjects 55 years of age or over The

Table III *Grading of symptoms in gastro-oeso-phageal reflux*

The symptoms are ranked on a scale from 4 to 1 Only one of the symptoms under 4 points is to be included in the sum of points for each individual

4 Points

Heartburn at rest or heartburn on bending forward or heartburn on exertion or regurgitation

2 Points

Lump in the throat epigastric tenderness excess of pha ryngeal mucus history of heartburn at end of preg nancy chest pain on exertion after meals

1 Point

Substernal pain provoked by exposure to cold substernal pain provoked by emotional stress other attacks of substernal pain attacks of hiccup attacks of air regur gitation cough

history of heart pain and the incidence of dysmotility of the oesophagus were far commoner among LES incompetent subjects than in those with LES competence

The smoking habits did not vary between the groups Full dentures were more often found in the group with LES incompetence All with full dentures showed either reflux or a disturbance of oesophageal motility or a combination of both

X ray

On X-ray examination four hernias were found Three of these were also demonstrated by manometry, and the fourth was diagnosed in a woman in whom manometry showed no pressure gradient between LES and the oesophagus

In 4 other subjects small hernias could not be excluded, but the findings did not quite fulfil the criteria as defined above Of these 4, one had a hernia, one showed reflux, and 2 showed normal findings in the manometric study Five of the subjects with LES incompetence had entirely normal X ray findings The hernias were demonstrated with the subjects lying down and during the swallowing of contrast medium with maximum extra abdominal pressure applied One hernia was also filled retrogradely when an external pressure of 60 mmHg was applied This subject had the largest hernia (6 × 6 cm), and was the only one in whom radiology disclosed reflux Valsalva manoeuvres did not elicit hernia in any case

DISCUSSION

It is probably more important to establish the diagnosis of pathological gastro-oesophageal reflux than a small sliding hiatal hernia The symptoms of a hiatal hernia are caused by regurgitation (Allison, 1951) and small hernias give regurgitation more often than larger ones (Sandmark, 1963*a*) Regurgitation and oesophagitis may also occur in the absence of clinical signs of hiatal hernia (Hiebert & Belsey, 1961) Furthermore, *hernias shorter than 2 cm are difficult* to demonstrate whether by X ray examination or manometry (Cohen, 1971) In the present investigation radiography disclosed reflux in only one of 11 patients shown to have [illegible] manometry This tallies with the experience [illegible] others (Hiebert & Belsey, 1961 Skinner [illegible] Since the history corresponded to the [illegible] *of LES incompetence* on manometric [illegible] tion, this technique for evaluating the [illegible] of the LES seems to be advantageous. With [illegible] 46 % (16/35) of the women and 69 % ([illegible] the men invited agreeing to take part, one [illegible] wonder how many of the objectors also [illegible] oesophageal dysfunction Even supposing [illegible] all the objectors had a perfectly normal [illegible] there would still be a female preponderance [illegible] respect to LES incompetence (26 ± 10 % [illegible] pared with 6 ± 3,7 % in the male subjects).

All pathological radiological findings were [illegible] accordance with the history and results of [illegible] metry *This is probably explained by the* [illegible] criteria used for diagnosing hernia If [illegible] signs to differentiate a small hernia from a [illegible] *phrenic ampulla had been used*, more her[illegible] might have been correctly diagnosed This [illegible] probably also have led to false positive [illegible] however, since the signs of gastric folds [illegible] the hiatus and obliteration of the incisura [illegible] and differences in motility between the phr[illegible] ampulla and a herniated part of the ventricle [illegible] sometimes difficult to identify *The difficul[illegible]* of distinguishing normal from pathologica[illegible] border line cases are illustrated by the 4 s[illegible] jects in whom a small *hernia could not be* [illegible] cluded Of these two were pathological and [illegible] were *normal* on manometry It is also of in[illegible] *to note that only* one of the diagnosed her[illegible] became retrogradely filled with contrast med[illegible] This may be explained by Sandmark's th[illegible] that heavy contrast *medium might draw the* [illegible] tricle into the abdomen and reduce a [illegible] hernia (Sandmark, 1963*a*) *The reason wh[illegible]* nevertheless *succeeded* in demonstrati[illegible] hernias when the patients *were actually small* [illegible] ing could be that this part of the examina[illegible] was performed *during* the first swallows, be[illegible] *any appreciable amount of contrast medium* [illegible] entered the ventricle

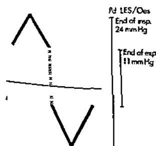
Since only one of the 11 *subjects with L*[illegible] incompetence was shown to have *reflux at rad*[illegible]

hy, and since 5 of them had entirely normal y findings, the X-ray technique seems to be ior to manometry in demonstrating LES mpetence

'nnett & Atkinson (1966) claimed that the na pectoris was more often due to a dis- r of the oesophagus than of the heart Koch (1964) observed a striking relationship be- n gastric, oesophageal, and heart disorders results show a more frequent history of ma pectoris' in the subjects with LES in- petence than in the others Concomitant ise of other organs innervated by the vagus e may be explained in several ways It may lue to misinterpretation of the symptoms, affections of the heart and the oesophagus, xample, are readily confused Another rea may be a common neurological factor

has been shown that smoking briefly lowers LES pressure (Dennish & Castell, 1971, eiu & Bennett, 1972) In this investigation ubjects were asked to refrain from smoking urs prior to the investigation No difference noking habits was seen between the 'reflux' the 'normal group

ie function of the LES is regulated by several rs of 'humoral and physical" character idrix, 1972) Our findings regarding the luminal pressure variations in the cardia in the oesophagus form a basis for a discus- of the possible sphincteric function of the ia No morphological proof has been forth- ing that LES is a sphincter It may be re- ed as such on functional grounds, however,



.4 The pressure gradients between the LES and the phagus in the LES-competent subjects (n 29) The es represent mean values The zero pressure point s to the intragastric pressure at the end of expiration

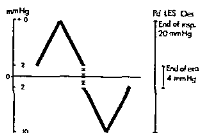


Fig 5 The pressure gradients between the LES and the oesophagus in the LES incompetent subjects (n−11) The figures represent mean values The zero pressure point refers to the intragastric pressure at the end of expiration

as the intraluminal pressure in the cardia can be influenced by various drugs and hormones (Castell & Lauran, 1970), like all tubular organs equipped with smooth muscle Presumably these smooth muscle cells in the submucosa are neces sary to close the lumen of the LES Beyond this closing mechanism, a valve mechanism based on the transdiaphragmatic pressure gradient augmenting the competence of the LES seems reasonable on the following ground During inspiration the fall in the intra oesophageal pressure is accompanied by a rise in the intra cardial pressure (Figs 4 and 5) The increasing pressure gradient during inspiration will cause a dynamic compression of the closed tube in analogy with the findings in the trachea of children suffering from an acute attack of croup (Ingelstedt et al, 1967) The valve mechanism which increases the competence of the LES during inspiration is easily seen on X-ray exam- ination of the oesophagus, and has been dis cussed by Creamer et al (1959) In order to overcome the dynamic compression of the cardia and to force it to open it is necessary to apply positive pressure exceeding the pressure gradient between the cardia and the oesophagus In order to cause reflux from the ventricle to the oeso- phagus, the gastric contents must be forced through the cardia In our series the mean intra gastric pressure of 10 mmHg, lagging behind the extra-abdominally applied pressure by one tenth, seems to have been operative in distinguishing between a competent and an incompetent LES, since the pressure gradient between cardia and

oesophagus in symptomless subjects, in contrast to those with symptoms, exceeds 10 mmHg at end of the expiration (Table I)

CONCLUSIONS

1 In a population selected at random women aged 55 years and over strongly predominate with respect to LES incompetence
2 The methods of X-ray examination used in this investigation are not reliable for demonstrating gastro-oesophageal reflux
3 The symptoms can be graded and the total score used in screening for oesophageal dysfunction
4 The competence of the LES is partly due to the dynamic compression obtained by the transdiaphragmatic pressure gradient
5 Gastro-oesophageal reflux takes place when the intragastric pressure exceeds the pressure differences between the LES and the oesophagus

ZUSAMMENFASSUNG

Mittels einer manometrischen Methode wurde bei einer wahllosen Auswahl von vierzig Personen in elf Fällen — überwiegend bei Frauen — eine Inkompetenz des unteren Ösophagussphinkters (LES) gefunden Die durchschnittliche Druckdifferenz zwischen LES und dem distalen Teil des Ösophagus bei Exspirationsende betrug 4 2 mmHg bei den LES inkompetenten Patienten und 10,5 mmHg bei den übrigen Sechs von den LES inkompetenten Patienten hatten einen Hernie-Hiatus Dysmotorik des Ösophagus überwog bei den LES inkompetenten Patienten Bei rontgenologischer Untersuchung fand man bei einem der elf LES inkompetenten Patienten einen gastroosophagischen Reflux und bei vier Patienten einen Hernie-Hiatus Alle der LES inkompetenten Patienten ausser einem hatten eine Anamnese die auf einen Reflux hindeutete

Die Symptome wurden nach einem Punktsystem gradiert Die Differenz der Punktsumme zwischen den LES kompetenten und -inkompetenten Patienten ist statistisch dreisternig signifikant ($p < 0\,001$) Die Symptome bei anderen Organen die vagusinnerviert sind, wurden auch zwischen den LES kompetenten und inkompetenten Patienten verglichen

Diskutiert wurde auch der Ventilmechanismus des Ösophagus

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IN VITRO ANTIBODY PRODUCTION OF HUMAN TONSIL FOR SHEEP RED CELLS (SRC)

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Abstract An attempt to determine the capacity of lymphocytic suspension from human tonsils to produce antibodies in vitro against sheep red cell was conducted using Marbrook s culture technique Detection of the antibodies was made using Cunningham s plaque forming method It was confirmed that the plaque forming cells increased predominantly on the 6th day of culture There was no relationship between the age of the patient and the plaque forming capacity The immunological roles of the human tonsil with additional reference to E and EAC binding lymphocytes previously reported become more pronounced

Recent advances in immunological techniques have suggested that human tonsils, as well as regional lymph nodes play an immunological role

In 1973, Tabata et al reported that subpopulations of lymphocytes from human tonsils were capable of rosette formation with both sheep red cells (SRC) and SRC sensitized with anti SRC antibody-complement It was then postulated that the human tonsil may well supply two different kinds of lymphocytes possessing immunological properties, such as do the thymus and bone marrow derived cells in mice (Bianco et al , 1970, Lay et al , 1971) In humans, therefore, the tonsils may perform a similar function to that of lymph nodes and produce in vitro antibodies for certain antigens

The present experiment is an attempt to determine the immunological function of the human tonsil by evaluating the capacity of tonsil lymphocytic suspension to form antibodies against sheep red cells

MATERIAL AND METHODS

Experimental materials were obtained fro[m pa]tients with a preoperative diagnosis of [hyper]trophied tonsils due to recurrence of c[hronic] inflammation There were 3 females aged [...] 9 years and 6 males aged 5 to 11 a total [of] patients with an age range of 5 to 11 [...] (Table I) The patients were otherwise f[ree of] systemic disease or complicating factors [...] the many advanced immunological techn[iques] available for in vitro antibody production [...] plaque forming technique using SRC as a[n ...] was selected for use in this experiment

Preparation of lymphocytic suspension from human tonsils

Cell suspension was prepared from sur[gically] removed palatine tonsils and was gently p[...] on the surface of 15 ml of 6% Angio[...] Ficoll The cell suspension was then centri[fuged] at 1 500 rpm for 10 min to separate the ly[mpho]cytes A confirmation of 90% lymphocyte [con]tent in the lymphocytic layer remaining [in the] test tube was made

Preparation for culture

Marbrook s technique for in vitro cultur[e was] slightly modified (Fig 1) and used as a g[...] procedure RPMI 1640 synthetic medium [...] supplemented with 10% of fetal calf serum [...] γ/ml streptomycine, and 100 unit ml pe[nicillin]

e I *Clinical description of patients*

men	Patient	Sex	Age
	K M	♀	7
	T Y	♀	9
	T T	♂	6
	K F	♀	7
	T I	♂	6
	T S	♂	5
	T F	♂	11
	T K	♂	8
	T K	♂	11

od was buffered with sodium bicarbonate 2×10^7 cell suspension was placed into the r culture compartment containing 1 0 ml lture medium, and SRC were then added mixed The lower compartment with 9 ml lture medium was effectively shielded with lysis membrane A control culture without en was prepared and placed in the upper artment The culture compartments were d in a humidified incubator at 37°C in an sphere of 5 % CO_2 in air

en

preserved in Alsever's solution were ughly washed with Hanks balanced salt ion before use The optimum dose of SRC ers was determined

Detection of antibody production by plaque-forming cells (PFC)

The plaque forming capacities of the cell suspension of the in vitro culture with SRC were detected according to Cunningham's direct method of hemolytic plaque assay for 19S antibody-producing cells (Fig 2) An outline of this procedure is summarized in Fig 3

Cell viability

Trypan blue staining (0 5 %) was used to detect the viability of the culture cells The percentage of survival cells per 200 cells was then determined

RESULTS

Cell viabilities were observed in the cultures for a period of 10 days (Fig 4) There was a 40–50 % survival rate of cells throughout the culture period An apparent decrease of viability occurred on the second day

Optimum dose of SRC antigen was determined on the 4th day of cultures of the tonsil cell suspension in good condition Table II demonstrates the plaque forming capacity of 2×10^7 tonsil lymphocytes with in vitro stimulation due to different concentrations of SRC on the 4th day of culture Five cases showed an increase of the plaque forming cells (PFC), in a case of 10^8 of SRC added A maximum number of PFC was 557 Control culture without

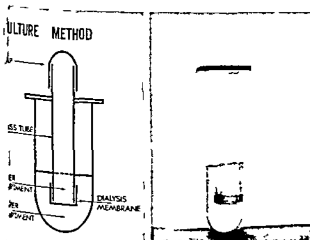


Fig 1 Schematic drawing of culture apparatus and the photograph

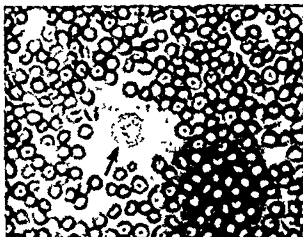


Fig 2 An arrow indicates plaque forming cell Phase contrast 10 × 40

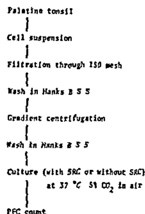


Fig 3 An outline of general procedure

antigen, however, showed a marked lower capacity to form plaques From this preliminary experiment, it was confirmed that optimum dose of SRC was 10^6 cells

Table III demonstrates the time course of plaque forming capacity in 4 cases The tonsil cell suspensions of 2×10^7 were cultured with 10^6 of SRC There was a prominent increase in plaques from Day 4–6 of culture in all specimens, although with individual differences The highest value was found on Day 6 of culture in the case of a five year old male, with a reading of 1332 ± 169 No correlation between patient age and capacity to form plaques was observed

DISCUSSION

In 1963, Ambrose successfully devised a method of culturing rabbit lymph nodes for the in vitro production of antibodies An improved culture technique was also reported by Ortiz-Muniz & Singel (1967) Surjan & Surjan (1968–1970) attempted an in vitro study of antibody production in rabbit tonsils and postulated that rabbit tonsil may take part in the immunological process Tabata et al (1973) reported no in vitro response of rabbit tonsil to antigens such as diphtheria and bovine serum, although peripheral lymph nodes produced antibodies, and concluded that rabbit tonsil plays a role of antigen messenger but not of antibody produc Apart from in vitro study of rabbit tonsils investigations on the immunological fun of the human tonsils exist because of the culties of immunological and in vitro c techniques

Surján et al (1971) reported that the h tonsils immunized against diphtheria, per and tetanus toxoids were able to produce body by tissue culture technique using ap priate unit of phytohemagglutinin (PHA stimulants The use of PHA, however aff antibody titer as determined by the hemag nation test Our previous experiments revealed that PHA, even in low concentrat

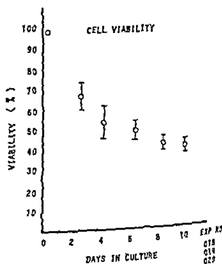


Fig 4 Graphical representation of cell viability thr out culture days

le II *The determination of optimum dose of igen responsible with 2×10^7 cell suspen- of human tonsils*

indicate the plaque-forming cells

No. of specimens				
011	012	013	014	106
23	5	157	66	68
—	—	122	80	60
91	73	360	122	165
111	271	557	124	201
62	237	389	90	73
27	—	193	32	55

ed non specific hemagglutination of sheep ls, therefore, the use of PHA should be For this type of study, more advanced unological and culture techniques will be ired in order to obtain the convincing results present study is an attempt of in vitro production of human tonsil using the rn techniques such as Marbrook's culture Cunningham's hemolytic plaque assay The plaque technique for detecting anti- synthesis at the cellular level described by & Nordin in 1963 was recently improved ingham & Szenberg (1968)

m's improved plaque technique may be of value in future experiments Using s culture technique for lymphocyte culture, a 40–50% cell viability was ed SRC was selected as the antigen plaque assay To insure results, im concentration of SRC was checked

III *Plaque-forming cells per 10^7 according of culture in cell suspension of human s 2×10^7 with added SRC antigen 10^6*

No. of specimens			
018	019	020	021
41 ± 11	10 ± 5	8 ± 4	11 ± 4
42 ± 18	14 ± 7	26 ± 5	13 ± 3
362 ± 97	209 ± 40	204 ± 56	159 ± 16
1332 ± 169	249 ± 54	193 + 39	314 ± 120
690 ± 243	278 ± 128	142 ± 20	119 ± 60
613 ± 362	78 ± 47	117 ± 26	78 + 21

It was determined that 10^6 SRC was an optimum dose to 2×10^7 tonsil cells

The occurrence of plaque forming cells observed in control cultures without antigen is thought to be related to a non specific reaction between tonsil cell suspension and SRC A similar finding was found in the original report by Marbrook (1967), although the mechanism of non specific reaction is still unknown In the cell culture with SRC antigen, the most predominant plaque-forming capacity occurred around 6th day and continued through the experimental period The result concords with a usual pattern of antibody production Antibody production against SRC appears to have no relationship to age of subjects used in the experiment Although the present experiment demonstrated the capacity of human tonsil lymphocytes to produce antibodies against sheep red cells, response to other bacterial toxins and allergens remains to be determined If suitable culture conditions can be established, further studies on these factors will be attempted Another interesting point for future study would be an evaluation of cell-coordination in the production of antibodies in the human tonsil

SUMMARY

In vitro study for antibody production in human tonsils was performed by using the plaque-forming technique Results showed that human tonsil lymphocytes produce specific antibodies to sheep red cell antigen

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Ein Versuch die Fahigkeit der Lymphozytensuspension der menschlichen Tonsillen zu bestimmen, die den Antikorper in vitro gegen die Schaf Erythrozyte produziert, wurde mit der Marbrook–Kulturtechnik gemacht. Der Antikorper wurde bei der Cunninghams platte-bildender Methode entdeckt Es wurde bestatigt, dass die platte bildenden Zellen unter den besten Bedingungen am 6 Tag der Kultur erheblich zunahmen. Die Antikorper-

Produktionskapazitat war von dem Alter des Patienten unabhängig Die immunologische Rolle der menschlichen Tonsillen, denen die oben gesagte E- und EAC binden-den Lymphozyten hinzugefugt wurden, hat sich überraschend geoffenbart

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LONG-TERM MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL EFFECTS OF SMALL MECHANICAL LESIONS IN THE GUINEA PIG COCHLEA

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bstract This paper reports the results on chronic mor iological and electrophysiological effects of small sions in 6 guinea pigs The lesions were made on the ternal wall on one of the three scalae of the basal ochlear turn Following a 6 week recovery period the ound window was exposed using a postauricular pproach and a platinum iridium ball electrode was aced on the round window Measurements of the CM ere made at ten frequencies Following completion of e electrophysiological measurements, the animals were rfused with Prussian blue solution and their temporal ones removed, fixed and stained decalcified and epared for microscopy In general electrophysiogical responses of the cochlea were depressed following iscrete mechanical lesioning Morphologically there as little influence segmentally limited on the vascu ture at the site of the lesion Little histopathological fect was seen on the organ of Corti Although no onsistent relationship was found between individual echanical lesions primarily restricted to one scala and bsequent electrophysiological effects findings of this udy do indicate that restricted mechanical damage to e cochlea results in restricted histopathology and a stricted effect on cochlear function

n a previous paper (Hallen et al, 1974), we escribed the immediate effects of small mechanical lesions made in the external wall of the uinea pig cochlea The effects of these lesions ere studied with both electrophysiological and orphological techniques Although we did not bserve any quantifiable and orderly correlation etween size and location of lesion and change in cochlear activity, it was noted that restricted lesions resulted in restricted changes of both electrophysiology and morphology of the cochleae The trauma caused by the mechanical lesion was limited to a narrow segment of the external wall with major changes restricted to the surrounding vessels No histopathological changes were seen in the organ of Corti

The authors concluded that the combined use of both electrophysiological and morphological techniques provides an effective and appropriate approach for the evaluation of the effects of surgically induced cochlear lesions We feel that such a combined approach provides distinct advantages over an approach based upon either of those measures alone The purpose of the present investigation is to extend these observations on the effect of inner ear surgery and trauma Specifically, the long-term effects of such mechanical lesions on the cochlear microphonic (CM) activity and histopathology of the cochleae are presented

METHODS

Subjects

A total of thirteen young adult guinea pigs weighing between 250 to 400 grams were used in this study Additionally, 18 guinea pigs were used as a control group of animals in this study for establishing normal CM activity No lesions were made in these animals All animals appeared healthy and exhibited a normal

his research was supported in part by NIH grant S 08181 and Office of Naval Research (Contract 00014-67 A-0103-0031) the Swedish Board for Techcal Development (project Fr 1500 u 1170) Gotaverken yard Company and Swedish Medical Research d (no B74-12X 558-10A)

Preyer's reflex After initial observation of middle ear infection in our first preparations, the animals were placed on ampicillin prophylactically for 2 weeks Electrophysiological and morphological information was obtained on 6 animals Morphological information only was obtained on 7 animals

Surgical procedure

Chronic lesions Each animal was anesthetized with sodium pentobarbital (35 mg/kg) and placed in a nontraumatic head holder A small incision was made in the neck which ran inferiorly 1 5 cm lateral to the thyroid gland The tissue and the trachea were gently retracted and the auditory bulla was identified The bulla was opened exposing the middle ear space and the cochlea Lesions were restricted to the basal turn and located about one-fourth of a turn from the basal end halfway between the round window and the bulla wall The bony wall of the cochlea was thinned with a small diamond drill A delicate pick was then used to remove the remaining bone Surgical trauma of the periotic and membranous labyrinth and intracochlear vessels was accomplished with a microdissecting knife in all animals except one, in which a cauterized lesion was made using an insulated electrode with a 5 μm diameter uninsulated tip The lesions were left without sealing the cochlear wall and the wound was sutured

Electrophysiology All electrophysiological recordings of the CM were made from the round window following a 6-week recovery period The animals were anesthetized with 0 2 % Penthrane and placed in a head-holder The external ear was removed and the auditory bulla was exposed using a post-auricular approach The bulla was opened, exposing the middle ear and bony capsule A platinum iridium ball electrode was placed on the round window, secured with dental cement and the bulla was sealed

Electrophysiological procedures

Electrophysiological procedures used in this paper have been described elsewhere (Hallen et al, 1974, McPherson & Miller, 19[illegible] one-half inch condensor microphone [illegible] earspeaker (PDR 600) were coupled [illegible] external auditory meatus The probe tu[illegible] advanced until it was 2 mm lateral [illegible] tympanic membrane The sound sour[illegible] probe tube were sealed such that the so[illegible] presented in a closed system

Measurements of the CM were m[illegible] frequencies of 0 08, 0 20, 0 50, 1 0 2[illegible] 6 0, 10 0, 20 0, and 30 0 kHz. In additio[illegible] 1 μV isopotential contour, the input [illegible] (dynamic range) functions of the CM [illegible] amined for a variety of frequencies A [illegible] Radio wave analyzer was used to rec[illegible] output of the CM

Histological procedures

Following completion of electrophysi[illegible] measurements, the animals were perfus[illegible] Prussian blue solution as previously de[illegible] (Axelsson, 1968, 1972, Hallen et al, 197[illegible] temporal bones were then removed a[illegible] cochleae fixed and stained with 0 5 % os[illegible] buffered with Millonig The bones were [illegible] fied in 10 % EDTA buffered to 7[illegible] cochleae were then washed, dehydrat[illegible] transferred to glycerin The temporal [illegible] were dissected with the aid of a di[illegible] microscope Sectioning of the cochlea [illegible] done so that portions of the externa[illegible] spiral lamina, and basilar membran[illegible] mounted on slides and the vasculature [illegible] neurepithelium, and supporting structur[illegible] examined under light and phase mic[illegible] Details of this preparation have been d[illegible] elsewhere (Axelsson et al, 1974)

Of the 13 animals subjected to surge[illegible] showed secondary complete fibrosis [illegible] cochlea, apparently resulting from labyr[illegible] One animal had a circumscribed laby[illegible] in the basal part of the scala tympani [illegible] site of the lesion Only those 6 animals [illegible] both electrophysiological and morpho[illegible] findings were achieved are presented her[illegible] morphological findings in the additi[illegible] animals were in agreement with those pre[illegible]



1 Animal I (318) Modiolus and most apical part of a vestibuli, basal turn, apico-basal section In a ment corresponding to the lesion (limits | -|), there four radiating arterioles of which one is completely jected (small arrow), two are lightly injected (the al ones) and one is well injected Other radiating rioles (*RAL*) are well injected There is a gap in "glomeruli" of the modiolus (large arrow) cor onding to the lesion There is a delicate collecting le running spirally in the most apical part of the a vestibuli



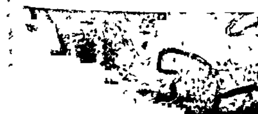
Fig 3 Animal I (318) Scala media basal turn, apico-basal section Basally to the lesion there is a part of the *external wall which is less well stained and where some* of the capillary loops in the stria vascularis are less well contrast injected

y abnormal findings are reported Omission comment indicates that electrophysiological morphological features were characteristic the normal guinea pig

mal No 1 (318)

ery An elongated fistula was made apically he scala vestibuli just beneath the second and three radiating arterioles were divided re was some leakage of perilymph

Morphology The hole in the bulla was almost led with the remaining hole sealed by a thin mbrane There was a bony ridge from the bulla wall over to the basal turn Pathological *periosteum vessels were noted in this region* A small bony protuberance was noted at the site of lesion The lesion appeared to have affected at least four radiating arterioles of which one was seen to be well injected and three were less injected with a gap in the "glomeruli" and in the apical part of the scala vestibuli in the area corresponding to the lesion and also basally to the lesion (Fig 1) Some capillaries in the basal part of the scala vestibuli (Fig 2) and in the apical part of the stria vascularis corresponding to the lesion were uninjected (Fig 3) The vessel of the spiral prominence was comparatively poorly injected in this region In the region corresponding to the lesion, the attachment of Reissner s membrane in the external wall was less prominent than in other regions and the membrane was folded there There appeared to be only minor changes in the organ of Corti with some indication of scattered early degeneration of outer hair cells restricted to the region of the lesion



2 Animal I (318) Basal part of scala vestibuli and d part of scala media basal to the lesion, apico-basal on *There is* a moderate gap in the vasculature sponding to the lesion with two uninjected radiating - (arrows) This part of the spiral ligament and vascularis (*SVS*) is less well stained

Electrophysiology There was a slight increase *in sensitivity of the 1 μV isopotential curve for* low frequencies (Fig 4) The dynamic range for 2 000 (Fig 5) and 4 000 Hz (Fig 6) showed a slight change in the slope of the dynamic range, however, the maximum amplitude was within the range of the control animals The input-output function at 10 000 Hz (Fig 7) was within the normal range

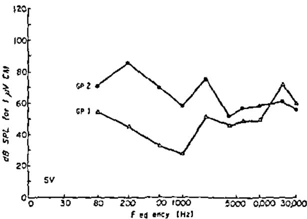


Fig 4 Animals 1 2 (318 343) Change in the 1 µV sensitivity contour, following lesion restricted to scala vestibuli The shaded area shows 2 S D about the mean curve for a control group of animals

Animal No 2 (343)

Surgery The wall of the scala vestibuli was thinned and a small fistula made immediately below the second turn There was some leakage of perilymph Two radiating arterioles were divided mechanically

Morphology There was a large bony overgrowth at the site of the lesion in the basal turn with some apparent pathological vessels on the outside of the cochlea There was also a arrow gap in the radiating arterioles in the

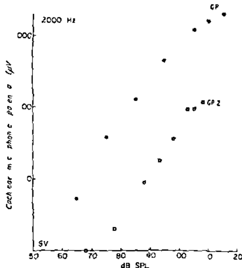
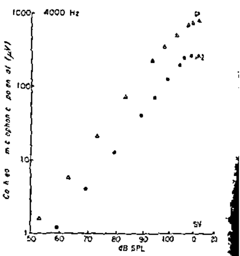


Fig 5 Animals 1, 2 (318 343) Input–output function of the cochlear microphonic at 2 000 Hz for lesion made in scala vestibuli The shaded area represents 2 S D about the mean value for a control group of animals



about the mean value for a control group of a

most apical part of the scala vestibuli prob affecting two radiating arterioles The bran in the basal part of the scala vestibuli w however, contrast injected Two or t radiating arterioles lack the normal sm appearance of the vessel wall (Fig. 8) T was a decrease of *pigmentation* in the s vascularis corresponding to the lesion and capillaries were less well injected here attachment of the vestibular membrane

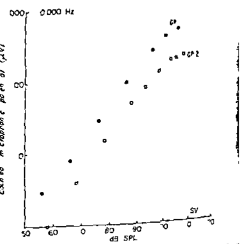
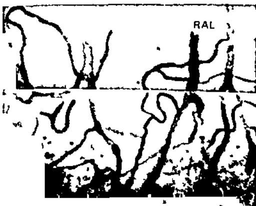


Fig 7 Animals 1 2 (318 343) Input–output func of the cochlear microphonic at 10 000 Hz for lesion in scala vestibuli The shaded area represents 2 S about the mean value for a control group of animals



8 Animal 2 (343) External wall scala vestibuli and la media apico-basal section Basally to the lesion the scala vestibuli the radiating arterioles (RAL) l capillary branches in the basal part of the scala stbuli are contrast injected (right half of picture) e vessel wall, however, seems more uneven than rmal and the capillaries somewhat dilated The illary loops in the stria vascularis (*SVS*) are less well cted



9 Animal 3 (344) External wall scala vestibuli d scala med a apico-basal section Apical to the lesion rge horizontal arrow) four radiating arterioles (*RAL*) w empty vessel lumen (see Fig 10) To the left of the ion, parts of the vestibular membrane lie folded nall arrow) There is thinning of the spiral ligament ally to the lesion particularly prominent in the stria (*SVS*) (see Fig 11)

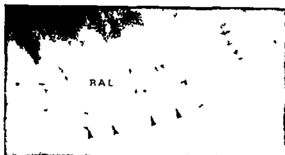


Fig 10 Animal 3 (344) Scala vestibuli apical to the lesion apico basal section Four of the radiating arterioles (*RAL*) show empty vessel lumen (arrows) Vessel walls are evident on each side of each arrow

more prominent and uneven than usual and there was an increased amount of pigment in the membrane corresponding to the lesion On the whole, there seemed to be little influence on the vascular pattern of the external wall Degeneration of outer hair cell stereocilia was observed restricted to the lesion site that did not appear due to preparation artifact

Electrophysiology There was a decrease in the sensitivity of the 1 μV isopotential curve for this animal (Fig 4) This was true for both low and high frequency with maximum change occurring at 2 000 Hz (25 dB)[1] The average decrease in sensitivity was approximately 20 dB The high frequencies 20 000 and 30 000 Hz were within normal limits as well as the lower limit of 80 Hz The dynamic range at 2 000 Hz (Fig 5) showed a marked reduction as well as a reduction in the dynamic range at 4 000 and 10 000 Hz (Figs 6 and 7)

Animal No 3 (344)

Surgery A small fistula was made in the scala vestibuli just at the junction to the second turn A mechanical lesion was made seemingly limited to one single radiating arteriole

Morphology The endosteum vessels in the basal turn of the cochlea were uninjected corresponding to the point of lesion There was a bony ridge going over from the bulla wall to the scala vestibuli and scala media in the basal

[1] Decibel change is computed from the mean value of the control animals.



Fig 11 Animal 3 (344) Scala media basally to the lesion apico basal section There is a narrow segment in the stria vascularis (*SVS*) with pigment deficiency and empty capillaries

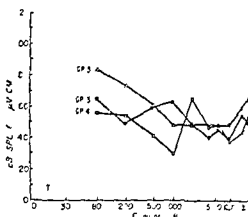


Fig 12 Animals 3 4 5 (344 338 323) Change in the 1 μV sensitivity contour following lesion restricted to scala tympani. The shaded area shows 2 SD about mean curves for a control group of animals

turn and the lesion was completely healed with bone There was a gap in the vasculature of the external wall apparently emanating from four or five radiating arterioles (Figs 9 and 10) At the level of the lesion there was narrow avascular gap in the stria vascularis and a narrow segment of deficient pigmentation (Fig 11) Arterio venous anastomoses passed this region Remnants of the vestibular membrane were irregularly folded at the site of attachment There were no large influences on the organ of Corti However in the area of the lesion the stereocilia in the second row of outer hair cells appeared comparatively distorted or absent

Electrophysiology The 1 μV isopotential contour for this animal showed a loss of sensitivity in the mid frequencies between 500 and 2 000 Hz (Fig 5) A maximum loss of 35 dB occurred at 1 000 Hz The input output function at 4 000 and 10 000 Hz appeared to be within the normal (Figs 6 and 7) There was a significant reduction in the input output function at 2 000 Hz (Fig 5)

Animal No 4 (338)

Surgery A small fistula was made in the scala tympani Moderate leakage of perilymph was noted Two collecting venules were divided and there was approximately 10 seconds of bleeding

Morphology The membranous external wall was detached from the bone at the lesion and bulged somewhat medially The lesion in the basal part of the scala tympani was completely healed by bone On the inside of the external wall there was an accumulation of blood at the site of lesion and two or three large collecting venules and corresponding finer branches were uninjected The vascular pattern of the scala vestibuli and media was in all respects normal

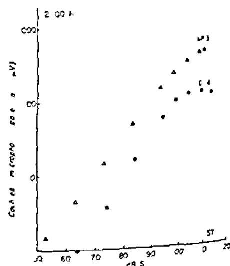
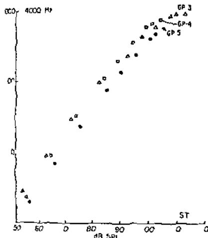


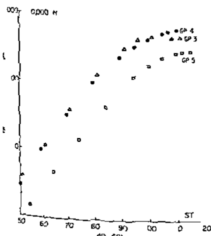
Fig 13 Animals 3 4 5 (344 338 323) Input-output function of the cochlear microphonic at 2 000 Hz lesion made in scala tympani The shaded area represents 2 SD about the mean value for a control group of animals



ig 14 Animals 3 4 5 (344 338 323) Input–output function of the cochlear microphonic at 4 000 Hz for lesion made in scala tympani The shaded area represents SD about the mean value for a control group of animals.

was the pattern in the scala tympani except the site of the lesion

Electrophysiology The 1 μV isopotential contour was normal for both low and high frequencies (Fig 12) The only frequency in which a significant difference occurred was at 2 000 Hz The loss at 2 000 Hz was 22 dB The input–output function at 2 000 Hz (Fig 13) showed a markedly reduced dynamic range The input–output functions at 4 000 Hz (Fig 14) showed a slight change



ig 15 Animals 3 4 5 (344 338 323) Input–output function of the cochlear microphonic at 10 000 Hz for lesion made in scala tympani The shaded area represents SD about the mean value for a control group of animals



Fig 16 Animal 5 (323) External wall basal turn apico-basal section The lesion (framed area) affects a large collecting venule in the basal part of the scala tympani (*ST*) (see also Fig 18) Almost all collecting venules (*CVL*) are well injected corresponding to the lesion Only two show deficient contrast injection (see Fig 17)

in the slope of the function but the maximum amplitude remained within that seen in the control animals The input–output function for 10 000 Hz was within normal limits (Fig 15)

Animal No 5 (323)

Surgery An elongated fistula was made basally in the scala tympani Moderate leakage of perilymph was noted Three collecting venules were divided mechanically There was approximately 20 seconds of bleeding into the scala

Morphology The lesion appeared to be completely healed by bone The lesion affected one of the largest branches of the vein of the round window (Fig 16) There was very little influence on the collecting venules in this region Some collecting venules appeared very clearly closed down at the Y formed junction of two branches and the empty continuing channel was easily observed (Fig 17) The large collecting

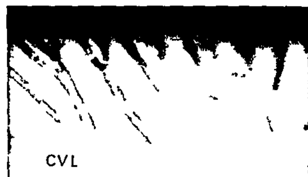


Fig 17 Animal 5 (323) Scala tympani basal turn apico basal section Corresponding to the lesion most of the collecting venules (*CVL*) are well contrast injected Only two collecting venules apically to the right of the lesion show uninjected common parts where two branches unite

venule in the middle of the lesion had a fairly uneven wall and large diameter (Fig 18) Single, uninjected radiating arterioles were also noted in the scala vestibuli In the scala media, there appeared to be no influence on the vessels The stria vascularis was fairly well injected and so was the vessel of the spiral prominence There were no gross changes in sensory or neural structures

Electrophysiology The isopotential curve (Fig 12) indicated a decrease in sensitivity for frequencies below 1 500 Hz with the maximum loss of sensitivity occurring at 1 000 Hz The magnitude of this loss was approximately 16 dB The higher frequencies likewise showed decreases in the 1 μV sensitivity however, the order of magnitude is less than that in the lower frequencies Input output function at 4000 and 10 000 Hz (Figs 14 and 15) showed a slight decrease in the dynamic range as compared to the control series of animals



Fig 18 Animal 5 (323) Scala tympani basal turn apico-basal section The lesion affects one large collecting venule The vessel lumen is intact but lacks the normal smooth appearance

Animal No 6 (322)

Surgery A wide exposure was made of the basal turn of the cochlea A fistula was made extending from the basal part of the spiral ligament almost to the level of the scala vestibuli A microelectrode was introduced into the stria vascularis and 20 μA were passed through the electrode for 3 seconds This electrolytic lesion was subsequently repeated immediately adjacent to the site of the first lesion

Morphology At the site of the lesion, there was a heavy bony overgrowth to the bulla wall This overgrowth originated in the middle of the stria vascularis and appeared to include the second and third turns A small hole remained in the external wall at the lesion An obvious gap in the vascular pattern of the external wall was noted with a corresponding segmental degeneration of the nervous structures in the spiral lamina (Fig 19) There was a pigment deficiency and reduced s

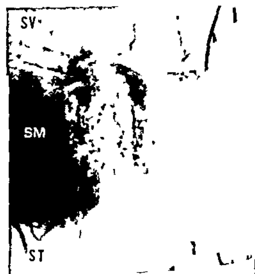


Fig 19 Animal 6 (322) External wall, apicobasal section The electric lesion affected the scala vestibuli (*SV*) and scala tympani (*ST*) to a limited extent and the scala media (*SM*) to a great extent Vessels are well injected on both sides of the lesion where there is a segmental narrow gap in the vasculature

~ the external wall in this region due to -arning and thinning out of the tissues The -ripheral marginal vessels in the spiral lamina -re less contrast injected at the level of the aon than elsewhere Sensory and supporting -uctures of the organ of Corti were completely generated in the area of the lesion In this gion, the basilar membrane was covered with -uamous epithelium tissue Likewise, a few ne fibers were seen to extend in an aberrant urse across the basilar membrane Phagocytic -ls were also evident (Fig 20) Degeneration -afferent myelinated nerve fiber in the modiolus s evident (Fig 21)

-Electrophysiology The 1 μV isopotential rve for this animal showed a significant -rease in sensitivity of the CM as a func- n of frequency (Fig 22) The greatest loss curred at 1 000 Hz The magnitude of loss at -s frequency was approximately 100 dB The -rage loss as a function of frequency was 50 Due to the magnitude of the loss, it was not ssible to obtain input-output functions for -s animal (The sound pressure level required -ceded capabilities of the transducer)

DISCUSSION

-ilarly to the previous investigation of acute mals, the current investigation presents



: 20 Animal 6 (322) Spiral lamina basal turn -sverse section. The organ of Corti adjacent to the -m s absent. A few nerve fibers seem to extend -n aberrant course over this region Phagocytes are -seen here Focusing at a lower level squamous -tal "scar" tissue is seen covering the basilar mem -e



Fig 21 Animal 6 (322) Spiral lamina basal turn transverse section Adjacent to the lesion there is a segmental degeneration of sensorineural and supporting structures There is a complete degeneration of the organ of Corti and myelinated afferent fibers in the modiolus The vessel injection is slightly impaired centrally and much impaired peripherally in this segment

observations on only a few animals Reservations concerning conclusions based upon such a small sample must be raised Some findings were similar and many were different from the previous examination

It was possible to make both large and small mechanical lesions in the external wall of the guinea pig cochlea without necessarily inducing labyrinthitis (one diffuse, one circumscribed in the thirteen animals) High power magnification of the operating microscope allowed easy visualization of the pigmentation of the stria vascularis and the borders between the three

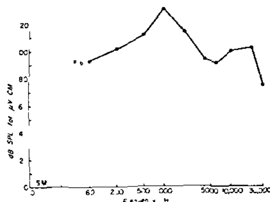


Fig 22 Animal 6 (322) Change in the 1 μV sensitivity contour following electrolytic lesioning of the scala media The shaded area shows 2 S D about the mean for a control group of animals

scalae of the external wall during surgery With the mechanical lesions, there was often some leakage of perilymph and short term hemorrhage, which under visual observation always subsided within a minute or two In the only case of the present material where electrical current was used for the lesion, the tissue seemed to be coagulated and there was no visible bleeding This lesion had a considerably more pronounced effect All mechanical lesions were healed by bone In the case of the electrolytic lesion, there was a remaining hole in the cochlea which was sealed only by fibrous tissue With either of these approaches, anatomically discrete lesions may be made in the cochlea

In most animals the bony structures in the bulla wall and the cochlear wall showed a pronounced tendency to heal Often the bony healing was abnormally thickened with bone protruding from the cochlea and also in many cases bony bridges formed between the bulla and the cochlear wall In all cases except one, the intracochlear structures of the external wall had assumed approximately their normal position

Findings of this study indicate that restricted mechanical damage to the cochlea results in restricted histopathology and a restricted effect on cochlear function These results in the chronic preparation (6 weeks survival post-surgery) corroborate previously reported anatomical and electrophysiological findings on the short-term effects of such lesions (Hallen et al , 1974) and long term anatomical findings (Axelsson & Hallen, 1973)

The histological finding of contrast present in the cochlear vessels indicates the presence of a patent vascular lumen It must be emphasized that the finding of contrast does not necessarily indicate a normal circulation in vivo since the contrast injection constitutes an artificial means of demonstrating the vessels Our experience from both injected and uninjected vessels, however, has convinced us that there is a relationship between the outcome of the contrast injection and the in vivo condition of the vasculature The effects on the vasculature were almost invariably limited to the external wall The findings in the present investigation by comparison to short term observations in the acute preparation, indicate that some vascular healing process follows these lesions In the short-term experiments, scala vestibuli lesions always caused a gap in the vasculature, both centrally and peripherally to the lesion In the present investigation, the three animals with scala vestibuli lesions showed only restricted influence on the vasculature at the site of the lesion and its immediate vicinity In one of these animals, there was an additional thinning of a narrow segment of the spiral ligament with some influence on the vessels in the stria vascularis In the other two, both the basal part of the scala vestibuli and the scala media demonstrated an approximately normal appearing vasculature

The only scala media lesion of the present investigation was the electrolytically induced lesion This injury was considerably larger both initially and in its end result The effect of this lesion on cochlear vasculature was similar to the large mechanical lesions presented previously (Axelsson & Hallen, 1973)

The two scala tympani lesions had similar effects on the vasculature and were similar to the effects observed in the acute preparation There was no influence on the vasculature in the scala vestibuli or scala media It thus appears that large lesions do not show secondary complete vascular healing but that they show a segmental limitation with the effect on the vasculature limited to a narrow gap (Axelsson & Hallen, 1973) With small lesions, as in the present investigation, and a fairly short survival time, the vascular repair process seems considerably more pronounced and the lesions are not only limited in segmental space but also limited to the immediate surroundings of the lesion These findings indicate a surprising healing tendency in the sensory organ

These small lesions had less histopathologic effect on the sensorineurepithelium and supporting structure of the organ of Corti than expected It was only in the case of the electrolytic lesion that clear degeneration occurred

nd in this case, in addition to the effect on structures of the organ of Corti, the extent of myelinated fiber degeneration was quite dramatic In all animals receiving the mechanical lesions alone, *the only indication of some* effect on organ of Corti structures was occasional distortion or absence of stereocilia in outer cells

On the basis of the electrophysiological observation, five points may be discussed

(1) There appeared no consistent relationship between individual lesions primarily restricted to one scala and subsequent electrophysiological effects For example, similar lesions restricted to the scala vestibuli resulted in different degrees of electrophysiological depression In general, no exact correlation was observed between the form and extent of the electrophysiological depression and specific features of the form and extent of histopathological changes These observations hold for both animals examined acutely, immediately after surgery (Hallen et al, 1974) and chronically, following a 6-week survival time This lack of relationship cannot simply be attributed to some immediate generalized disruption of cochlear function Our inability to determine the exact relationship between anatomical and electrophysiological changes induced by these lesions may, however, simply be due to the small sample size examined to date [1]

It is clear that on a gross level, a relationship exists between organ of Corti destruction and depression of the *cochlear response The most* dramatic change in this study was when a microelectrode was introduced into the scala media and an electrolytic lesion created A dramatic decrease was seen in the 1 μV isopotential contour This occurred for all frequencies Also, morphological findings were severe within the area adjacent to the lesion where a complete elimination of the organ of Corti and the development of epithelial scar tissue along the basilar membrane was observed *It is difficult to compare this scala media lesion* with the mechanical lesions placed in the other scalae in this group of animals However, the observation of a greater effect with the scala media lesion than observed with lesions of the other scalae, is consistent with our findings on the short term effects of such surgeries It is also consistent with the findings of Tasaki et al (1952) The only condition under which they noted changes in the electrophysiology of the cochlea was when lesions were made in the scala media We suggest that this may be due to the fact that lesions in the scala media have a direct effect on the longitudinal distribution of the CM in the cochlear partition Certainly in regards to other investigations on the generation of the CM (Honrubia & Ward, 1968), such an explanation is reasonable

(2) In assessing cochlear function, it appears that the input–output function of the CM may be more sensitive to cochlear changes than an examination of the 1 μV isopotential contour This was a consistent observation in animals examined in this study These observations agree with previous findings of the effects of experimental manipulation on cochlear activity (McPherson & Miller, 1972, 1974)

(3) By comparison to observations on the immediate effects of mechanical lesions to the *lateral wall of the cochlea (Hallen et al, 1974)*, the somewhat longer term effects examined in this investigation appear somewhat more restricted Again, limited sample size employed in these two studies places some reservations on such comparisons

(4) One of the most important observations in this study is the fact that healing occurred after discrete lesioning of the cochlear wall, resulting in relatively small changes in the electrophysiological response of the cochlea This is particularly encouraging in that the changes seen electrophysiologically in this study were less severe than those seen where the short-

[1] Reservations regarding the sensitivity of the measures used in these studies were discussed in the previous paper (Hallen et al, 1974) In particular, as suggested by others (Bohne et al, 1973, Bredberg 1968) we feel low power evaluation of organ of Corti structures permitted by light and phase contrast microscopy is of insufficient sensitivity to reflect the functional state of these structures except in the case of marked destruction

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term electrophysiological effects of such lesions were examined Encouraging also is the continued observation of the restricted nature of anatomical and electrophysiological effects of such restricted lesions

(5) Only small electrophysiological changes occurred in association with those surgeries in which slight perilymphatic leakage and/or bleeding was encountered However, moderate or excessive bleeding and leakage was associated with a clear and, in many cases, severe depression of the cochlear potentials Apparently, perilymphatic leakage at the time of surgery, in which the only cochlear damage was a small mechanical lesion of the lateral wall, may serve the surgeon as a cue for both the immediate and more long term surgical influence on cochlear function

It should be clear that we place many reservations upon the observations made in this study, principally because of a small sample size of subjects examined with this approach to date Additionally one must be concerned about the sensitivity of such measures as round window recordings of electrical activity of the cochlea and light microscopy for the examination of stopathological changes However, we do gest that the combined electrophysiological al approach taken in this study is a fruitful one for studies of this nature We feel that at this time it is important to determine the effect such inner ear surgery has on the status of the cochlea Such observations are significant in light of the increasing interest by otolaryngologists on the possibility of performing vascular and electrode implantation surgeries on the cochlea of man

ZUSAMMENFASSUNG

Die chronisch morphologischen und elektrophysiologischen Einwirkungen von kleinen Schaden bei sechs Meerschweinchen werden mitgeteilt Die Schaden wurden an der ausseren Wand in einer der drei Scalen von der basalen Schneckenwindung gemacht Sechs Wochen spater wurde das runde Fenster mit Hilfe eines postaurikularen Zuganges exponiert und eine Platinum Iridium Ball Elektrode wurde am Fenster angelegt Messungen der CM wurden mit 10 Frequenzen vorgenommen Nach den elektrophysiologischen Messungen wurden die Tiere mit Berlinerblau perfusiert und die Temporalknoche wurden ausgenommen fixiert und gefärbt, und für Mikroskopie praparert Im allgemeinen … die elektrophysiologischen Antworten von der Coch nach den mechanischen Schaden niedriger Morph gisch wurden nur wenige und immer segmental begrenz Einwirkungen an den Gefassen in der Gegend de Schadens beobachtet Das Cortische Organ w histopathologisch sehr wenig beeinflusst

Die Befunde dieser Untersuchung deuten darauf h dass begrenzte mechanischen Schaden an der Cochl auch begrenzte histopathologische Effekte und ein begrenzte Einwirkung auf die Cochleafunktion ergeb Keine regelmassige Verhältnisse zwischen und mekanischen Schaden die zu einer Scala begränzt w und die darauf folgenden elektrophysiologischen Effek wurden beobachtet Dagegen scheint es als ob ein Zu sammenhang zwischen Destruktion vom Cortisch Organ und Depression der cochlearen Antworten und elektrolytische Schaden in der Scala media exsist

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SYNAPTIC VESICLES IN THE COCHLEA

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(Received February 15, 1974)

Abstract The cochlea is innervated by afferent, efferent and sympathetic neurons The afferent synapses and the efferent and sympathetic nerve terminals contain transmitter substances within their synaptic vesicles These transmitters are likely to be chemically different in each of the three cochlear nerve populations The character of intra-cochlear synaptic vesicles was investigated in order to throw some light on transmitter identity Their staining properties were examined using different fixatives and after pretreatment with false transmitter substances and a monoamine depleting drug The efferent and sympathetic cochlear terminals were identified and differentiated by surgical ablation of either the efferent olivo-cochlear bundle or by cervical sympathectomy The nature of these terminals could also be ascertained in unoperated animals but only after pretreatment with false transmitters or after special fixation techniques In this way a catecholamine appeared to be the transmitter of the sympathetic nerve-terminals The staining properties of cochlear afferent synaptic vesicles and synaptic bars made it unlikely that a catecholamine was the transmitter Vesicles in efferent terminals reacted similarly to vesicles in cholinergic neurons

The existence of vesicles in the presynaptic part of neurons was first discovered by De Robertis & Bennett (1954) and Palade & Palay (1954) Since then several types of vesicle have been described Most nerve endings contain small clear vesicles clustered in the vicinity of the presynaptic membrane They are found in both cholinergic neurons and in neurons containing other transmitters, for example biogenic amines and amino acids

A special type of synaptic vesicle containing a dense core was discovered by Hager & Tafuri (1959), De Robertis & Pellegrino De Iraldi (1961) and Richardson (1962, 1966) Small vesicles with a diameter of about 500 Å and large vesicles around 1 000 Å in diameter can occur together

Electron microscopic evidence now exists that catecholamines are contained in dense-cored vesicles (Richardson, 1962, 1966) This has been confirmed by Hökfelt (1968) using an *in vitro* technique involving incubation in monoamine-containing solutions and fixation with potassium permanganate There is good evidence that both large and small dense core vesicles in adrenergic axons contain a monoamine or its precursor (Klein & Turesson-Klein, 1971) Administration of false transmitters and the action of certain drugs has verified this conclusion Cholinergic neurons also contain large dense cored vesicles but they are not affected by the above-mentioned substances and are not considered to be involved in the monoamine metabolism It is thought that these vesicles as well as so called coated vesicles and large clear vesicles, which also exist in most nerve endings, are involved in the process of recovery of membrane and related material (Akert & Sandri, 1970, Heuser & Miledi, 1971)

The complicated innervation pattern in the cochlea and organ of Corti has been extensively studied From the electron microscopic work of Engström (1958) and Engström & Wersäll (1953, 1958) two types of nerve ending, the vesiculated and the less vesiculated were identified Vesiculated nerve endings were assumed to be efferent in nature (Engström, 1958) The path-

The investigation has been supported by grants from the Swedish Medical Research Council, no. 04x 2461, and from the Karolinska Institute

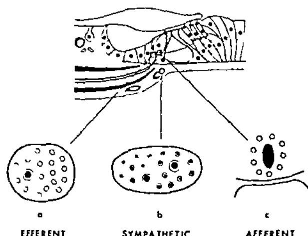


Fig 1 Schematic drawing illustrating typical location for synaptic vesicles of different types (*a*) efferent terminals of the olivo-cochlear bundle (*b*) sympathetic nerve endings (*c*) the afferent synapse between inner hair cells and sensory nerve fibre

ways of the efferent innervation were orginally described by Rasmussen (1946, 1960) in cat and has also been identified in the rabbit (Borg, 1973) Further investigations on the efferent innervation in the cochlea have been performed by Kimura & Wersäll (1962), Smith & Rasmussen (1963), Spoendlin & Gacek (1963) and Iurato et ' (1971)

The cochlea also receives a sympathetic ...ation, which can be demonstrated with the Falck–Hillarp (Falck et al, 1962) fluorescence technique for catecholamines (Terayama et al, 1965, 1966 Vinnikov et al, 1966, Spoendlin & Lichtensteiger, 1966, 1967) and by electron microscopy (Densert, 1974) As far as the afferent nerve fibres are concerned the hair cells are the presynaptic elements—they contain synaptic vesicles at their base and are likely to transmit chemically

The aim of the present study was to investigate synaptic vesicle populations in the organ of Corti by using surgical elimination and different fixation methods in order to find distinguishing features which can facilitate the identification of different synapses and terminals, and perhaps throw some light on the question of transmitter identities

Particular attention was paid to efferent and sympathetic, adrenergic nerve endings in the region of the osseous spiral lamina and the afferent synapse at the base on hair cells.

MATERIAL AND METHODS

Fourteen white rabbits weighing from 1 2 to 3 0 kg were included in the present investigation. The following procedures were used

A *Surgical Procedures*

1 *Sectioning of the olivo cochlear bundles*

In 3 animals, under Nembutal® anaesthesia, the skull was opened in the occipital region. The cerebellum was elevated slightly Using a sha knife, at an angle of 45° to the fourth ventri a 2–3 mm deep incision was made through the restiform body, immediately behind the dors cochlear nucleus Both the crossed and uncrossed olivo-cochlear fibres, as well as the reticulocochlear fibres, were thereby interrupted as they pass along the intracerebral part of the vestibul nerve Bilateral lesions were produced Fi to 9 days after sectioning the olivo-cochlea bundle, the animals were decapitated and the brain stem was removed and fixed in 10% formalin After embedding in paraffin, the specimens were sectioned and stained accordin to Kluver & Barrera The cochleas were fixed in osmium tetroxide as described below (B1) or by potassium permanganate (B2)

2 *Sympathectomy*

In 2 animals unilateral extirpation of the superior cervical ganglion was performed under Nembutal® anaesthesia 1 and 2 weeks befo sacrifice In 2 other animals, where the ol cochlear bundle was sectioned unilateral cerv sympathectomy was also performed 5 OH D as described under C1, was administered to o animal in each group before decapitation

B *Fixatives Used*

1 1% osmium tetroxide in veronal aceta buffer (Rhodin, 1954), post mortem or b perfusion *in vivo*



2 A low power view of a section through the brain n showing bilateral surgical lesions through the olivo-hear bundle Stained according to Kluver and Barrera

3 % potassium permanganate in 0 1 M sodium phosphate buffer at pH 7 0 for 45 minutes (Luft, 1956, Richardson, 1966, Hokfelt, 1968)

3 % glutaraldehyde (0 133 M sodium phosphate buffer, pH 7 3) followed by 1 % osmium tetroxide

C *Pretreatment with Drugs*

The false transmitter 5 OH DA (3,4,5 trihydroxyphenylethylamine hydrochloride) was ministered intravenously or by local perfusion ation was carried out in potassium permanate as described under B2 5 OH DA was ministered i v 4 × 20 mg/kg over a period of hours (Tranzer & Thoenen 1967) For local fusion the tympanic bulla was opened and a all hole was made to the scala tympani with ard of a diamond drill The stapes was ached and the cochlea was perfused via the lled hole for 5 minutes with a Ringer solution taining 0 1 mg/ml 5-OH DA

2 6-OH DA (2,4,5 trihydroxyphenethylamine hydrochloride) was administered i v or via local perfusion before fixation in osmium tetroxide according to B1 6-OH DA 50 mg/kg was administered i v ½ to 3 hours before sacrificing the animals Local perfusion with 6-OH DA was performed in the same way as for 5 OH DA, but to the solution was added 0 2 mg/ml ascorbic acid

3 Reserpine (10 mg/kg) was administered 24 hours before sacrifice Fixation was performed in potassium permanganate or glutaraldehyde and osmium tetroxide according to B2 and B3 respectively

Fixed specimens were dehydrated in ethanol and embedded in Epon (Luft, 1961) Ultrathin sections were cut with a diamond knife on an LKB ultrotome The sections were stained in uranyl acetate (Reynolds, 1963) and lead citrate (Watson, 1958) except for specimens fixed in potassium permanganate which were only briefly stained in lead citrate The sections were examined in a Siemens Elmiskop I electron microscope

RESULTS

Surgical Procedures

When the ipsilateral superior cervical ganglion was removed all adrenergic nerve terminals and fibres in the cochlea degenerated One week after the sympathectomy no adrenergic structures were identified around the spiral vessel of the tympanic lip and in the habenula region, where they were normally abundant in untreated animals (Fig 1) In other parts of the cochlea there were no signs of nerve fibre degeneration

When the olivo cochlear bundles were sectioned (Fig 2) all vesiculated nerve endings of efferent nature under the inner and outer hair cells, like nerve fibres in the inner spiral bundle and tunnel spiral bundle (Figs 3, 5) degenerated About 2 weeks later it was not possible to identify any efferent axons in the cochlea (Figs 4, 6)

In two experiments unilateral *sympathectomy* was performed in addition to bilateral total olivo-cochlear ablation The cochlea on the sympathectomized side did not contain any vesiculated endings, whereas the remaining vesiculated nerve endings in the cochlea on the other side were of typical adrenergic appearance and location

Vesicle Appearance in Different Fixatives

Osmium tetroxide

Fixation in 1% osmium tetroxide gave good preservation of ultrastructural details in the cochlea Efferent and adrenergic nerve terminals were filled with synaptic vesicles having *similar appearance* (Figs 7, 8) Thus, it was not possible to differentiate between adrenergic and efferent terminals according to the number or size of synaptic vesicles or by other ultrastructural features The afferent synapse in hair cells also contained this type of vesicle (Fig 15) Most vesicles had a diameter of about 300–400 Å, although some were as large as 1 000 Å in diameter All synaptic vesicles were spherical As a rule, small vesicles appeared devoid of electron dense material, while large vesicles in both efferent and adrenergic terminals contained a rather dark, electron dense core The synaptic bar at the afferent synapse was al... tron dense A few adrenergic synaptic v... might contain a small dense core These cored vesicles were more apparent whe... mens were fixed by *in vivo* perfusion of ... There was no apparent difference in properties of efferent terminals when fixation was performed either *in vivo* or minutes after death

Glutaraldehyde

When the specimens were fixed in glutaralde... and postfixed in osmium tetroxide s... synaptic vesicles in efferent endings and ... cells appeared empty and were devoid of electron dense material (Fig 9) A few s... *monoamine storage vesicles contained a d...* core (Fig 10) Large synaptic vesicles conta... a distinct dense core, both in efferent adrenergic terminals The density of the syna... bar in hair cells was similar to these dense co... The electron dense material almost filled vesicles, leaving only a narrow gap betwe... dense core and vesicular membrane L... dense-cored vesicles were abundant in both inner spiral bundle below inner hair cells a... the tunnel spiral bundle

Potassium permanganate

This is a strong fixative and the preserva... ultrastructural detail was inferior to that obta... with osmium tetroxide However syn... vesicles and terminals were fairly well preser... In efferent endings small vesicles appe... *empty and the large ones* contained an ev... distributed weakly staining material (Fig 11... adrenergic terminals a rather large numbe...

Fig 1 Electron micrograph from a normal animal ... are obs... spiral b... sium per... (TSB) P, inner pillar cell Fixatio... ganate × 10 900 ... olivo-cochlear bundle ...

CF
TSB
AF
AF
2 μ
jp
AF
AF
g
2 μ

:les contained a small but distinct dark core . 12) These dense cores were most evident in ll adrenergic vesicles Small vesicles in ent nerve terminals were devoid of an ron dense core Similarly, afferent synaptic :les did not contain a dense core and the ptic bar was barely visible

Pretreatment with Drugs

droxydopamine

n the animals were pretreated with 5-OH- accumulation of the substance was seen in nergic synaptic vesicles After fixation in *ssium permanganate* a distinct dense core eared in all monoamine-storing vesicles 13) Efferent synaptic vesicles, on the other d, did not change after pretreatment and no ease in electron opacity was observed icles at the afferent synapse did not contain ense core, nor did the synaptic body stain sely (Fig 14) The appearance of the afferent apse in an untreated animal is shown in Fig

fter fixation in *glutaraldehyde and osmium oxide* adrenergic synaptic vesicles contained osmophilic substance This black substance d both small and large vesicles Large aptic vesicles in efferent terminals contained istinct core but its electron density was not eased by 5 OH DA pretreatment

ydroxydopamine

en 6-OH DA was administered i v it accu- lated in adrenergic synaptic vesicles Within minutes most vesicles exhibited a dense core r fixation in osmium tetroxide Adminis- tion of 6-OH DA, however, was followed by a degeneration and the number of dense cored vesicles rapidly diminished with time The accumulation of 6-OH-DA in adrenergic vesicles was even more evident when the cochlea was perfused locally with a 6-OH-DA-containing solution (Fig 11) In efferent nerve terminals there was no visible accumulation of 6-OH-DA, nor were hair cells affected

Reserpine

When animals were pretreated with *reserpine* the dark core of adrenergic vesicles did not appear after fixation in potassium permanganate (Fig 17) and it was no longer possible to distinguish vesicles in adrenergic terminals from those in efferent endings or hair cells No change was seen in the appearance of the synaptic bar in inner hair cells

DISCUSSION

Transmitter substances and their identification by electron microscopy has attracted much interest since the discovery of storage vesicles in the presynaptic part of neurons (De Robertis & Bennett, 1954, Palade & Palay, 1954) The theory that acetylcholine is stored in vesicles was put forward by Del Castillo & Katz (1956) v Euler & Hillarp (1956) proposed a similar hypothesis concerning the storage of noradrenalin Considerable effort has been made to identify transmitter substances with electron microscopy Such identification has been possible only for biogenic amines

The ability to demonstrate monoamines at an ultrastructural level depends primarily on the fixation technique Osmium tetroxide was first used to demonstrate amine storage sites in peripheral neurons (Grillo & Palay, 1962, Richardson, 1962) Potassium permanganate, which was introduced as a fixative by Luft (1956), has turned out to be specific in demonstrating biogenic amines (Richardson, 1966) The reliability of this technique was further improved by Hökfelt (1968) The reaction between the fixative and the amine complex results in a precipitate, which

5 Electron micrograph from a normal animal, strating efferent nerve fibres travelling in the tunnel al bundle (*TSB*) and as tunnel crossing fibres (*CF*) erent fibres (*AF*) cross the tunnel at a lower level *IP*, er pillar cell × 8 400

6 After transectioning the olivo-cochlear bundle, fibres disappear, whereas afferent ones remain × 6 200


7

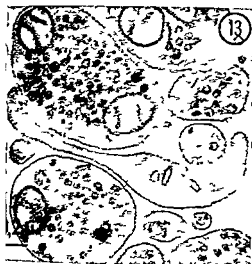

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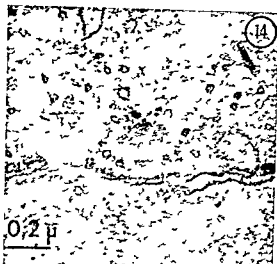

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13 Pretreatment with 5 hydroxydopamine and fixa n in potassium permanganate enhances the density the core in adrenergic sympatic vesicles × 43 500
14 The synapt c vesicles at the afferent synapse fail show a dense core in spite of pretreatment with ydroxydopamine and fixation in potassium perman late The synaptic body is weakly stained × 53 300

Fig 15 The afferent synapse in a non treated animal is shown after fixation with osmium tetroxide × 51 300
Fig 16 Adrenergic nerve endings in a cochlea perfused with a 6-OH DA solution Vesicles contain a black substance and degeneration has started Fixation in osmium tetroxide × 59 000

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7 Efferent terminals fixed with osmium tetroxide ta n small vesicles and a few large dense cored icles, × 25 700
8 Adrenerg c terminals fixed with osmium tetroxide e vesicles s milar to efferent ones × 27 700
9 Fixation in glutaraldehyde followed by osmium oxide Efferent terminals contain both small clear large dense-cored vesicles × 31 300

Fig 10 Fixation in glutaraldehyde followed by osmium tetroxide Adrenergic terminals have a few small granulated vesicles The large vesicles contain a dense core × 37 100
Fig 11 Potassium permanganate fixation does not alter the staining properties of efferent synaptic vesicles × 41 100
Fig 12 Potass um permanganate stains synaptic vesicles in adrenergic terminals in a characteristic manner small as well as large vesicles have a dense black core 37 300

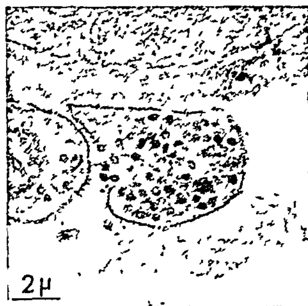


Fig 17 Pretreatment with reserpine depletes catechol am ne stores in adrenergic synaptic vesicles and they no longer exhib t a dense core × 54 000

is visible in the electron microscope as a dark, electron dense core in the amine storage vesicle The use of ' false transmitter substances such as α methylnoradrenalin (Bondareff, 1965, Hökfelt, 1968) and 5 OH DA (Tranzer & Thoenen, 1967) made it possible to increase the amine level in amine storage vesicles and make the demonstration of adrenergic neurons more reliable It has been established that small dense-cored vesicles in adrenergic neurons contain a monoamine and that the large dense cored vesicles take part in monoamine metabolism The existence of large dense cored vesicles in cholinergic neurons has been interpreted in different ways However, there is no evidence that monoamines are present in cholinergic neurons

In the present study the location of efferent and adrenergic nerve endings was established by surgical ablation of the olivo-cochlear bundles and by cervical sympathectomy The identification of the afferent synapse posed no problem

In the ultrastructural study of the inner ear, *fixation with osmium tetroxide or glutaraldehyde* followed by fixation in osmium tetroxide are the most commonly used techniques These methods give good ultrastructural preservation but efferent and adrenergic nerve terminals fixed with these agents, have almost the same appearance (Ross, 1973) After fixation in glutaraldehyde and/or osmium tetroxide a few small adrenergic vesicles contain a black core but this is not sufficient to ascertain the identity of the terminals When the specimens were fixed in potassium permanganate most amine storage vesicles appeared granulated, and their appearance was enhanced after *5 OH DA* pretreatment The existence of a monoamine in these granulated vesicles was further proved by their disappearance after pretreatment with reserpine

Synaptic vesicles in efferent nerve endings, in adrenergic nerve terminals and in afferent synapses in hair cells have been compared The staining properties of efferent synaptic vesicles are as would be expected in cholinergic neurons, a fact which agrees with the suggestion that acetylcholine might be the transmitter in the olivo-cochlear neurons (Schuknecht et al 1959

Nerve terminals in the habenula region and around the spiral vessel of the tympanic lip contain vesicles which have all the characteristics of catecholamine-containing neurons The presence of fluorescence specific for catecholamines in this region supports this concept (Densert 1974) Attempts have been made to identify transmitter substances in afferent synapses in hair cells Three theories as to the nature of the afferent transmitter have been suggested catecholamine (Osborne & Thornhill 1972) glutamate (Steinbach & Bennett, 1971) and γ aminobutyric acid (Flock & Lam 1974)

Since afferent synaptic vesicles and bars stain weakly in potassium permanganate and are not influenced by 5 OH DA, 6 OH DA and reserpine it seems unlikely that a catecholamine is involved

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ZUSAMMENFASSUNG

Die Cochlea wird von afferenten, efferenten und sympathischen Neuronen innerviert Die afferenten Synapsen sowie die efferenten und sympatischen Nervenendigungen enthalten Transmittorsubstanzen in ihren synaptischen Vesikeln. Diese Transmittoren sind wahrscheinlich chemisch verschiedene Substanzen in jeder der drei Cochlearnervpopulationen Die Beschaffenheit der intracochlearen synaptischen Vesikel wurde in Hinblick auf ihre färberischen Eigenschaften unter Anwendung verschiedener Fixierungsmittel und nach Vorbehandlung mit falschen Transmittorsubstanzen sowie einer Monoamin-depleerenden Substanz untersucht Die efferenten und sympathischen cochlearen Nervenendigungen wurden durch chirurgische Abtrennung des efferenten olivocochlearen Bundels oder durch cervicale Sympathektomie identifiziert und differenziert Die Natur dieser Nervenendigungen konnte auch an nicht operierten Tieren ermittelt werden, jedoch nur nach Vorbehandlung mit einem falschen Transmittor oder nach Verwendung von speziellen Fixierungstechniken In solchen Fallen erschienen Catecholamine in sympathischen Nervenendigungen, waren aber in efferenten Nervenendigungen nicht zu erkennen Die farberischen Eigenschaften der cochlearen afferenten synaptischen Vesikel und synaptischen Körper machten die Annahme unwahrscheinlich, daß ein Catecholamin als Transmittor wirkte

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PINOCYTOSIS IN THE PILLAR CELLS OF THE ORGAN OF CORTI

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stract Electron micrographs are presented showing nings in the head plates of the pillar cells of the organ Corti. The possibility of fluid transport between the nel-spaces and the subtectorial space is inferred m these observations The capabilities of the pillar ls for fluid transport were further examined through use of the exogenous tracer, Thorotrast®, which was seen in the tunnel-spaces until at least 40 min after roduction into perilymph After 2 hrs Thorotrast was nd in vesicles in all parts of the pillar cells and closely plied to the external membrane On the basis of a re- w of histochemical observations relating to the pillar ls together with morphological findings and results tracer experiments, it appears that these cells are im- ated in active transport processes involving perilymph, ndolymph, and the fluid of the subtectorial space They y help to maintain microhomeostasis in the cochlear nsducer

cent studies have produced evidence that cer in of the non sensory cells of the organ of rti play important roles in the transport of ids and macromolecules (de Lorenzo et al, 73, Lim, 1970*b*, Vosteen, 1970) During the urse of a previous study involving close scru- y of the reticular lamina in the guinea pig right & Preston, 1973), we found tiny open- gs, frequently surrounded by dense clusters of icrovilli, scattered on the head plates of the llar cells Further study of these structures ith both scanning and transmission electron icroscopy led to a series of exogenous tracer periments designed to investigate the active transport capabilities of the pillar cells The results of this work, together with histochemical and ultrastructural data from other investigations, suggest that, in addition to their function as structural supports, the pillar cells are capable of transporting substances between fluid compartments in the cochlea by pinocytosis or cytopempsis

METHODS

Our initial observations of openings in the pillar head plates were made in a series of 5 young adult guinea pigs (3 experimental and 2 control animals) used in a study of cochlear innervation (Wright & Preston, 1973) Cochleas from these animals were fixed immediately *post mortem* by the zinc iodide – osmium tetroxide (ZIO) method as described in the above publication Subsequently, the same structures, identical in appearance, have been seen in normal guinea pig cochleas fixed in 6% phosphate-buffered glutaraldehyde, or 10% cacodylate-buffered acrolein, followed by post-fixation in 1% phosphate-buffered osmium tetroxide (OsO_4) Both *in vivo* and rapid *post mortem* perfusion of the perilymphatic spaces was employed

Freeze drying for scanning electron microscopy (SEM) was based on the technique of Bredberg et al (1970) CO_2-critical point drying from amyl acetate-immersed, ethanol dehydrated tissue was also used as a check for freeze-drying artifacts Ears from several mammalian species in addition to guinea pig were prepared

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using similar techniques and surveyed for the presence of these structures in pillar cells

Colloidal thoria sol-dextrin (Thorotrast®) was introduced into the perilymphatic spaces by a method similar to that of von Ilberg (1968*a*, *b*) After surgical exposure of the cochlea both the round and oval windows were opened The perilymph was then replaced by Thorotrast by perfusion through either the round or oval window

Perilymphatic perfusion of Thorotrast was repeated several times during the course of each experiment Large amounts of Thorotrast were presented to the perilymphatic scalae to facilitate interpretation of its distribution in scala media at the end of the experiment Great care was exercised not to puncture the basilar membrane through the round window To terminate the Thorotrast experiments we perfused the cochlea with 6% glutaraldehyde, decapitated the animal and immersed the temporal bones in the fixative for 24–48 hrs before OsO_4 post-fixation, microdissection and Araldite-embedding according to conventional techniques

RESULTS

Electron-microscopic findings

Initial observations with SEM disclosed small openings in the head plates of the pillar cells (Fig 1*a–e*) These structures vary greatly in size (0 5–3 μm greater dimension) but are consistent in having smooth, raised borders, apparently composed of fusions of microvilli Suggestions of internal structure, again interpreted as being microvilli, are frequently visible (Fig 1*a*, *f*) Raised areas, blister-like in appearance, can also be seen, some of these suggest underlying channels (Fig 1*a*, *b*) Other structures, with accompanying rings of fused microvilli, brought to mind openings either recently closed or soon to be opened (Fig 2) When we were able to look into the tunnel, after dissecting away the basilar membrane and mounting the specimen in inverted fashion, we again detected openings in the pillar cell surfaces (Fig 2)

Having convinced ourselves of the validity of these observations after using three different primary fixatives and both criti freeze drying methods, we attempted to strate the structures using transmissi microscopy (TEM) We have been so far in finding an actual opening, membrane-bound channel, lined w in a pillar head plate is clearly show

Our current impression regard bution of the head plate openings in turns is that they probably occur uniformly from base to apex, althou h perhaps be more easily noticed third of the organ of Corti The in a given tissue sample varies widely f eral per pillar cell to only a few entire cochlear turn We have now structures in the chinchilla, guinea pig, tree shrew, and macaque (*Macaca*

Experiments with Thorotrast

The openings observed in pillar suggestive of pinocytotic activity, we undertook to learn more about the of the pillar cells for fluid transport exogenous tracer, Thorotrast A total of ments were done using this tracer fixed 20 and 40 min and 2 hours after the administration of the tracer in chinchillas, and 30 min, 2 hours (2 and 4 hours in 4 guinea pigs

In general the distribution of ThO_2 observed with TEM in our experimental is similar to that seen by von Ilberg (1968 and by Duvall & Quick (1969) Large of the tracer were visible, in all ears, in the stance of the basilar membrane spiral and spiral ligament and in vesicles in the

Fig 1 Scanning (*a–e*) and transmission (*f*) electron graphs of the guinea pig organ of Corti showing in the tops of inner pillar cells thought to be 710 fixa m glut ation F d to drying and coating tissue for *a–e* (*f*) Taken from a uranyl acetate citrate stained Araldite section *IH* inner hair *OHC* first row outer hair cell *ST* subtectorial Bars indicate 1 μm


IH
IH
OHC
ST

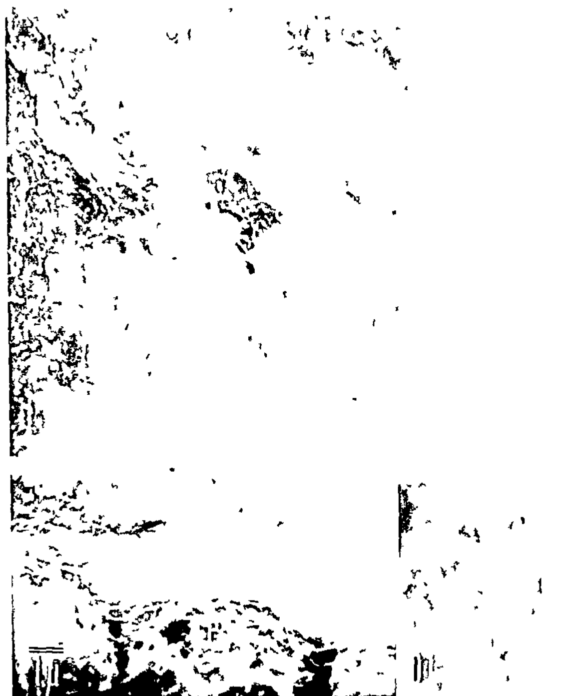
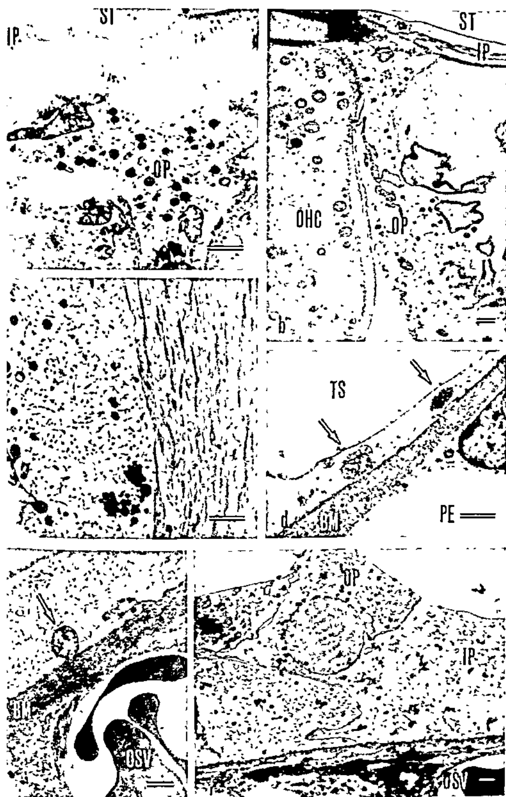


Fig 2 Scanning electron microscopic view of tunnel aspect of pillar heads showing openings (downward pointing arrows inset) in or between outer pillar cells Upward pointing arrows indicate ring arrangements of microvilli frequently seen in neighborhood of openings Guinea p g third turn OsO_4 fixation *IP* pillar cell *OP* stalk of outer p llar cell

Fig 3 Transmission electron m crographs of the organ of Corti apical turn from chinchillas showing the d stribution of Thorotrast which had been injected into perilymph 2 hours (*a–c*) and 40 min (*f*) before fixation Regions of pillar cells shown in each picture are described in the text The arrows indicate large ves cles in the p llar feet contain ng high concentrations of tracer apparently being taken up from the basilar membrane. *C* hyde-OsO_4 Arald te uranyl acetate *BM* basilar brane *IP* inner pillar cell *OHC* first row o t cell *OP* outer pillar cell *OS* outer sp ral vess perilymphatic space of scala tympani *ST* sub space *TS* tunnel space Bars ind cate 1 μm.


ST
IP
OP
ST
IP
OHC
OP
b
TS
d
BM
PE
BM
OSV
OP
IP
OSV

panic cover layer and in the fibrocytes of the spiral limbus On the other hand, no particles of ThO_2 were seen in the pillar cells or Deiters' cells or in the fluid spaces of the tunnel in the 20-, 30-, or 40 minute experiments (Fig 3*f*)

In the ears fixed 2 hours or more after beginning the administration of tracer, ThO_2 particles were observed in the pillar and Deiters cells and in the tunnel spaces in all sections examined The amount of incorporation of tracer varied greatly, however, from place to place in the cochlear spiral Since we were interested in the fate of the material after it had entered the tunnel, we chose to look at areas with maximal concentration of ThO_2 particles in the fluid spaces and supporting cells (Fig 3*a–e*)

Once the tracer material has entered the tunnel spaces, apparently by way of the supporting cell bases (Fig 3*d*, *e*), it is found uniformly distributed in vesicles in all parts of the supporting cells including the head plates (Fig 3*a*, *b*) and stalks (Fig 3*c*) of the pillar cells, and the phalangeal processes of Deiters' cells The material is also seen densely applied to the surface coats of supporting cells (Fig 3*b*) We have not observed ThO_2 particles either along the membranes or in the cytoplasm of the outer hair cells It appears frequently, however, surrounding the upper tunnel crossing nerve fibers and occasionally in the axoplasm of these fibers

Toxic reactions

In an effort to investigate the possibility of toxic reaction induced by the presence of Thorotrast, two additional ears were perfused with a 25% aqueous solution of potato dextrin The appearance of cytoplasm in various cell types did not differ appreciably between animals given Thorotrast or dextrin alone, from this observation we conclude that ThO_2 itself is not toxic to cells of the organ of Corti

In untreated guinea pigs the number of clear vesicles seen in tympanic cover layer fibrocytes and pillar feet was similar to that in the experimental animals, suggesting that pinocytotic activity is not generally stimulated by the addition of the dextrin containing preparation

It is, of course, admitted that some ad reactions may occur due to experimental c tions such as deprivation of perilymph cooling of the otic capsule in the exposed dition These appear to be minimal how within the time periods used in our experi and to be confined to slightly swollen chondria in the hair cells (visible in Fig. 3

In summary, the results of our tracer ex ments indicate no passive diffusion of Thor into the fluid spaces of the tunnel of Corti, none was seen there in experiments lasti min or less The amount of active transp the supporting cells varied unsystematically region to region of the cochlea in our e ments, however, and it remains a possi that in areas of high activity active tran could occur in a very short time Interact the part of the supporting cells by pinoc or cytopempsis (as discussed by Vosteen with perilymph near the cell bases and the of the tunnel and the Nuel spaces is cl demonstrated

DISCUSSION

There are few previous observations of a p morphological character which would lend port, or even hint at, the idea of a fluid tran function for the pillar cells Various pub electron micrographs including the fine exa of Angelborg & Engstrom (1972) demons the presence of mitochondria in adequate bers for oxidative metabolism related to a level of surface activity Mitochondria ar centrated in the cytoplasm of the pill (which contain the nucleus) and are clu in the stalks and heads between the to ments (stalks), terminal web (heads) and external membrane The horseradish per experiments made by de Lorenzo et al. (led him to suspect an active role on the p the supporting cells in the metabolism of C organ With SEM we have observed a su tial distribution of microvilli on the entir face of the pillar cells facing the tunnel (Fig 2), in addition to the openings d in this paper

Certain histochemical enzymological findings the literature can now be interpreted in light morphological observations made with and thout the benefit of exogenous tracers sternhagen (1970) found high concentrations protein bound sulfhydryl and disulfide groups the stria vascularis and pillar cells of guinea s, as demonstrated by the DDD reagent ese concentrations were reduced in animals fering from chronic experimental arsenic or rcury poisoning Energy metabolism involved transport processes would be expected to pond in this manner to heavy-metal poisoning zymes associated with oxidative metabolism, luding DPN and TPN-diaphorase and cyto rome oxidase, have been demonstrated on the face of pillar cells (Gerhardt, 1962*a*, *b*, Lim, 70*a*) Nakai & Hilding (1967) observed ATP- activity on supporting cells of Corti's organ were surprised at the presence of this enzyme the subtectorial region In our laboratory P ase has been shown to be specifically as ated with the pillar head plates (Thomas P rr, in preparation) Spoendlin & Balogh (1963) orted strong succinic dehydrogenase activity the upper parts of the Deiters' cells and re- rked that this finding suggests a functional ect more general than that of providing sical support for the sensory cells

Openings in pillar cell surfaces and actual nnels possibly involved in fluid transport are rly evident in our electron microscopic ob- vations although their apparent overall in quency of occurrence might lead us to ques their functional significance Since it appears, vever, that their formation and disappearance y be a dynamic process the small numbers n in some specimens need not force us to count the possibility of an important role ier evidence available to support the idea of ctional continuity between the fluid spaces ither side of the reticular lamina comes from electrophysiological measurements of Law e et al (1974) who were unable to demon te a difference in DC potential between the nel and the subtectorial fluids

Our failure to detect ThO_2 particles in the tunnel spaces in three animals with 20-, 30-, and 40-minute Thorotrast perfusions, makes it difficult to believe that passive diffusion actually occurs across the basilar membrane In these animals, moreover, the material was present in great concentration in the basilar membrane to the level of the basal lamina underlying the pillar and Deiters' cells Above this level, and in the tunnel the observed concentration of Thorotrast is smaller in the majority of sections examined even in tissue samples from the long term experi- ments The existence of a large concentration gradient across the membrane argues, by defi- nition, against passive diffusion as a transport mechanism This claim is at odds with the inter pretation of von Ilberg (1968*b*) restated by Vosteen (1970) that passive diffusion between the scala tympani and the tunnel spaces occurs, and that the tunnel fluid is actually perilymph Von Ilberg states (1968*b*, p 390) that after 30–60 minutes ThO particles were found on the cell surfaces lining the tunnel space a statement not incompatible with an active transport mechanism Although the electrolyte composition of the tunnel fluid is not yet known, and may be found to resemble closely that of perilymph, our conten tion that active transport occurs across the feet of the pillar cells is in accord with the con- cept of cortilymph as proposed by Engstrom (1960) and reviewed by Engstrom et al (1965)

We believe that the difference in apparent affinity for the tracer between hair cells and supporting cells which was consistently evident in our experiments may constitute a significant observation It is also noteworthy that ThO_2 particles were seen in high concentration opposed to the stria vascularis in cochleas in which large amounts of tracer entered the scala media This phenomenon is now being investigated and we are attempting to determine what surface charge, if any, exists on the colloidal particles in Thoro- trast which might explain the distribution of the substance over the membranes of various cell types in the organ of Corti

As this work proceeds we hope to learn more about the special properties of the supporting cells which permit them to play an important

role in the maintenance of microhomeostasis (Hawkins, 1973) in the cochlea

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ZUSAMMENFASSUNG

Es werden elektronenmikroskopische Aufnahmen gezeigt, die Öffnungen in den Kopfplatten der Pfeilerzellen des Cortischen Organs erkennen lassen Anhand dieser Befunde wird die Moglichkeit eines Flussigkeitstransports zwischen den Tunnelräumen und dem subtektorialen Raum erortert Die Fahigkeit der Pfeilerzellen, Flüssigkeit zu transportieren, wurde ausserdem mit Hilfe des Kontrastmittels Thorotrast untersucht Thorotrast konnte in den Tunnelraumen fruhestens 40 Minuten nach Einbringen in die Perilymphe nachgewiesen werden Nach 2 Stunden wurde Thorotrast in Vesikeln in allen Teilen der Pfeilerzellen sowie an der äusseren Membrane gefunden Nach Betrachtung histochemischer Beobachtungen an Pfeilerzellen, sowie aufgrund der morphologischen Befunde und der Ergebnisse der Thorotrast-Experimente hat es den Anschein, dass diese Zellen an aktiven Transportprozessen beteiligt sind, die die Perilymphe, Cortilymphe und die Flussigkeit des subtektorialen Raumes umfassen Diese konnen dazu dienen, die Mikrohomoostasie im Schneckenrezeptor aufrecht zu erhalten

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Na-K-ATPase ACTIVITY IN THE COCHLEA OF THE RAT DURING DEVELOPMENT

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act The development of the Na K-activated AT-enzyme system was studied in the stria vascularis is from 6–46 days post partum In 6–8-day-old ani rather high Na K-ATPase activity was observed eafter the activity increased gradually until the 12th A surprisingly sharp increase of the Na K ATPase ity was found from 12–18 days The adult level was ed between 19 and 25 days after birth, which coin- with the functional maturity of the cochlea These together with comparable data on the development e ERP and the ionic composition of the endolymph, rm the assumption that the Na-K ATPase system e stria vascularis is responsible for the maintenance e cation gradients and for the generation of the ERP

ny studies have been performed on the mor-logical and functional development of the hlea The majority of these experiments deals the anatomical development of the coch- structures in connection with the develop-nt of the cochlear potentials

nggård (1965) observed in rabbits a close tionship between the development of the hlear potentials, the morphological matura- of the cochlear tissues and the response to ustical stimulation In rats a relationship veen the development of the electrical activity the response to sound stimulation has been nonstrated by the experiments of Schmidt & nandez (1963), Crowley & Hepp-Reymond 66) and Bosher (1972)

Although many studies have been performed he adult cochlea on the relationship between metabolic processes in the cochlear tissues the electrical activity (Konishi et al, 1961, Konishi & Kelsey 1968, Bosher & Warren, 1968 Thalmann et al, 1973) up to the present, no such experiments have been performed during the ontogenesis of this organ

In view of the important role of the intra-cellular-like ionic composition of the endolymph in cochlear function, we studied in earlier experi-ments the distribution of the Na-K-ATPase activity in the adult organ In many tissues this enzyme system has been shown to be intimately connected or even identical with the cation pump system The Na K-ATPase system in the stria vascularis was concluded to maintain the cation-ic gradients between endolymph and perilymph or blood (Kuijpers & Bonting, 1969, Konishi & Mendelsohn, 1970) Moreover a close relation-ship between the functioning of this enzyme system and the existence of the endocochlear resting potential (ERP) was established In addi-tion a strong dependency of the cochlear micro-

The present study was undertaken to trace the development of the Na-K-ATPase activity during the ontogenesis of the cochlea in order to study its relation to the development of the cochlear function

METHODS

The experiments were performed on [illegible] rats of an inbred strain For dissec[illegible]

rats aged from 6–46 days (the average gestation time was 22 days) were killed by decapitation A reliable method for the dissection of the stria vascularis in animals younger than 6 days appeared to be impossible

After removing the lateral wall of the middle ear and the mucous substance, present in the middle ear space of the younger animals, the cochlea was exposed Subsequently the bony capsule of the cochlea was carefully removed The spiral ligament with stria vascularis of the lower two turns was detached from the modiolus and the stria vascularis dissected and lyophilized as described before (Kuijpers & Bonting, 1969)

ATPase activities were determined in homogenates of the stria vascularis (4 specimens for each assay) The methods used have been described earlier (Kuijpers & Bonting, 1969)

For histological studies the animals were perfused by intravitam perfusion with formaldehyde and, after decalcification, embedded in paraffin and sectioned

RESULTS

In preliminary experiments ATPase activities were determined in the tissue complex consisting of stria vascularis and spiral ligament However reproducible data could be obtained This failure was partly due to a variable amount of bone chips adhering to the spiral ligament, which

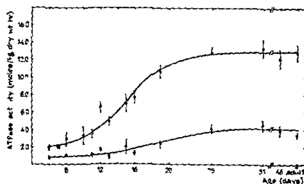


Fig 1 Mg (●) and Na K (○) activated ATPase in stria vascularis homogenates from rats at different ages Each point represents the mean value with S E of 4 determinations (MKH moles ATP hydrolyzed kg dry weight/hr at 37 C)

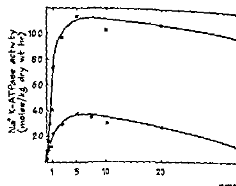


Fig 2 Effect of various K+-concentrations on th K ATPase activity in the presence of 60 mM Na and 25 days post partum

could not be removed in an adequate way Th fore the enzyme activities were measured o isolated stria vascularis

Fig 1 shows the Na K- and Mg act ATPase activities in the stria vascularis at d rent ages In the 6- and 8-day old animal ready a high amount of Na K-ATPase a was found (2 5 MKH) During the days this activity gradually increased How from 12–18 days after birth a very rapid inc of the Na-K-ATPase activity of the stria va laris occurred, from 4–10 MKH The adult of both Na-K- and Mg activated ATPase a ity was reached between 19 and 25 days partum Histological examination of the c lear structures at that age showed a m anatomical structure

Figs 2 and 3 show the activation by ca of the Na-K-ATPase system at 10 and 25

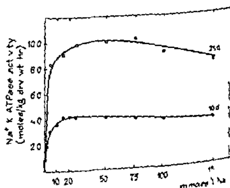


Fig 3 Effect of various Na+-concentrations on the K ATPase activity in the presence of 5 mM K+ and 25 days post partum

le I *Activities and properties of ATPase in cochlea of various animals*

	Guinea pig stria vasc.	Chicken tegm vasc	Rat stria vasc
ATPase activity (H)	7 0	8 0	13 0
ATPase activity (H)	5 6	12 4	3 8
maximal Na^+-conc)	4 5	4 9	2 0
maximal K^+-conc)	0 9	0 9	1 0

d moles ATP hydrolyzed (kg dry weight) hr at 37° C

r birth At both ages half maximal activation ached at 1 0 mM K^+ in the presence of 60 Na$^+$ and at 2 mM Na^+ in the presence of M K^+ Maximal activation was obtained at 30 mM Na^+ and 5 mM K^+

DISCUSSION

data presented in Fig 1 show that the AT- e activities in the stria vascularis of the rat h the adult level between 19 and 25 days r birth These data are in good agreement h the morphological maturation of the coch- r structures the maturity of the electrical entials and the response to sound stimulation hmidt & Fernandez, 1963, Crowley & Hepp- ymond, 1966, Bosher, 1972)

In Table I the data obtained on the ATPase ivities in the full grown cochlea of the rat presented together with identical data ob- ned in the guinea pig stria vascularis (Kuijpers Bonting 1969) and the tegmentum vasculosum omologous to the stria vascularis) in the chic- n for purposes of comparison (Kuijpers al, 1970) These structures have in common extremely high Na K ATPase activity com- red with other tissues in which cation trans- rt occurs against an electrochemical gradient onting, 1970) These findings in the rat give ditional support for our assumption that this zyme system in the stria vascularis and the mentum vasculosum plays a central role in the maintenance of the cationic gradients between the endolymph and the perilymph or blood

From the enzyme data obtained during the ontogenesis of the rat cochlear structures (Fig 1) it appears that the stria vascularis already at 6–8 days post partum showed a considerable amount of enzyme activity (2 5 MKH) Bosher (1972) demonstrated in 8-day old rats a nearly adult ionic composition of the endolymph, while the ERP was only a few mV In view of these findings it seems likely to assume that this amount of enzyme activity is able to maintain the intracellular like ionic composition of the endolymph at that age The further increase of the Na-K-ATPase activity from 4 0–10 0 MKH, observed between 12 and 18 days seems not to be essential for the maintenance of the cationic gradients, since no measurable increase in the size of the endolymphatic space was observed during this period In about the same period, between 12 and 15 days, Bosher (1972) observed a very sharp rise of the ERP from −20 to −85 mV, *without significant changes in the ionic* composition of the endolymph From earlier experiments a close connection between the functioning of the Na-K ATPase system in the stria vascularis and the existence of the ERP was established (Kuijpers & Bonting, 1970) The nearly simultaneous increase of the Na K-AT Pase activity and the ERP is in agreement with this assumption According to this hypothesis an increase of the ERP implicates that more K^+-ions had to be pumped into the endolymph, since the Nernst potential for K^+-distribution (figures for K -conc derived from Bosher, 1972) is −80 mV, a greater deviation from the Nernst potential requires more energy, i e an increase of the ATPase activity (Nernst potential $E = -(RT/F) \ln (K^+\text{endol}/K^+\text{peril})$ mV) Since no difference in the activation curves by Na^+ and K^+ are observed (Figs 2 and 3) before and after the sharp rise of the ERP it seems unlikely that this rise can be attributed to a change in the characteristics *of the enzyme system However, a con*tribution to this rise of the ERP by changes in the resistance of the membranes of the cochlear

duct as suggested by Bosher (1972) cannot be completely excluded

In conclusion it can be stated that these results confirm our former assumption that the Na-K-ATPase system in the stria vascularis is responsible for the maintenance of the cationic gradients between endolymph and perilymph or blood and for the generation of the ERP

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ZUSAMMENFASSUNG

Die Entwicklung des Na K aktivierten ATPase Systems wurde in der Stria vascularis von 6 bis 46 Tage alten Ratten untersucht. Bei 6 bis 8 Tage alten Tieren wurde *bereits eine ziemlich hohe Enzymaktivität gefunden* Nach einer langsamen Zunahme bis zum 12 Tag wurde eine sehr grosse Steigerung bis zum 18 Tag observiert Das erwachsenen Niveau wurde zwischen 19 und 25 Tagen post partum erreicht in derselber Zeit ist auch die Schnecke funktionell ausgewachsen Diese Ergebnisse zusammen mit vergleichbaren Daten zum Verhalten des ERP und des Ionengehalts der Endolymphe, stützen die Auffassung dass das Na K ATPase System in der Stria vascularis fur die Aufrechterhaltung der Kationengradienten und fur die Erzeugung des ERP, verantwortlich ist

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ADRENERGIC INNERVATION IN THE RABBIT COCHLEA

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act The distribution of adrenergic nerve fibres in ibbit cochlea was investigated with fluorescence and on microscopic techniques Interest was focused on ibenula region After administration of a false trans the fine structure of adrenergic nerve fibres and relationship to other structures were studied A tude of adrenergic nerve terminals were found id radiating nerve fibres where they lose their n sheaths Apart from this nerve fibre innervation was an extensive innervation of the spiral vessel of mpanic lip under the inner hair cell This blood l was of capillary size and lacked contractile ele- s. No adrenergic innervation was observed in the of Corti or in the stria vascularis The cochlear ergic innervation was found to have its origin in the teral superior cervical ganglion Possible ways of ergic influence on sound perception was discussed

adrenergic innervation of the inner ear has ved much attention since it was suggested several diseases of the inner ear, such as itus, vertigo, and sudden deafness, may be to an autonomic imbalance (Seymour & pin, 1951, Passe, 1953)

ound perception and blood circulation in the hlea have been investigated under various erimental conditions A sympathetic effect cochlear microphonics has been registered, the results have not been clearcut (Beickert al, 1956 Rambo et al, 1953, Seymour & pin, 1951) The morphology of the vascular as in the cochlea has also been studied but r function has been difficult to assess in tion to the endo- and perilymphatic fluids and the supply of the organ of Corti with oxygen and nutrients

Injuries to the stria vascularis do not seem to affect the organ of Corti, while obstruction of the spiral vessels causes a degeneration of the hair cells (Lawrence, 1966, Alford et al, 1965) Blood vessels in the stria vascularis and the spiral vessels have been observed during stimulation of the cervical sympathetic trunk No visible changes were seen in flow rate or diameter of these vessels (Perlman & Kimura, 1955)

As a basis for an investigation of the adrenergic influence in the cochlea, the morphology of the adrenergic innervation has first to be considered Earlier histological methods for light micro scopy, such as silver staining, were non specific and coloured all non myelinated nerve fibres and even connective tissue (Palumbi, 1954, Andrzejewski, 1955, 1956) Thus it was difficult to clearly distinguish adrenergic nerve fibres from other fibres By means of the fluorescence method specific for catecholamines (Falck et al, 1962), cochleas from cats (Spoendlin & Lichtensteiger, 1966, 1967) rabbits, rats and guinea pig (Vinnikov et al, 1966) and guinea pigs (Terayama et al 1965, 1966) have been investigated Vinnikov et al (1966) reported the existence of adrenergic nerve fibres in the inner spiral bundle, but apart from this, no adrenergic innervation was to be found in the organ of Corti or stria vascularis The cochlea is difficult to investigate

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because it is embedded in bone, and bony lamellae surround the spiral ganglion and nerve fibres radiating to the organ of Corti Terayama et al (1965, 1966) used cryostat sectioning and they claimed the innervation to be a pure blood vessel innervation ending at the small vessel under the inner pillar or the tunnel of the organ of Corti However, subsequent electron microscopic studies failed to demonstrate adrenergic nerve terminals around the spiral vessels beneath the inner pillar and tunnel or at the habenula perforata (Terayama et al, 1968) When specimens were treated according to the Champy-Maillet zinc iodide – osmic acid technique (Hawkins, 1968), light microscopy demonstrated "a vasomotor innervation by unmyelinated nerve fibres that accompany the myelinated cochlear fibres but descend to pierce the basilar membrane and terminate on the vessels" under the tunnel of Corti In stretch preparations, Spoendlin & Lichtensteiger (1966, 1967) found an innervation of blood ·s in the modiolus and an arcade-like habenular plexus independent of blood vessels No definite adrenergic innervation has been found distal to the inner hair cell

In an electron microscopic study on cats, where a false transmitter was administered, continuous blood vessel innervation was found extending as far as the spiral vessel of the tympanic lip A blood vessel independent plexus was found around nerve bundles in the habenula region and especially around the non myelinated part of the axon (Densert & Flock, 1974)

There are marked anatomical differences between cat and rabbit concerning the internal carotid artery, which is lacking or rudimentary in cat This is probably the reason why all sympathetic nerve fibres to the head pass the middle ear in cat

Because of its species differences, and since the rabbit is well suited for experiments in cochlear physiology it warrants a separate description The aim of the present study was to investigate the adrenergic innervation of the rabbit cochlea by means of fluorescence and electron microscopic techniques and by nerve sectioning, to try to find the sympathetic pathways to the inner ear

MATERIAL AND METHODS

In this study 19 white rabbits weighing from 1 2 to 4 0 kg were used

Fluorescence microscopy

Five normal rabbits were used In order to enhance the fluorescence, L-dopa (10 or 20 mg/kg i p) was administered to some animals one hour before decapitation In order to the monoamine stores, reserpine (10 mg/kg i p) was administered 24 hours before sacrificing In 2 animals the tympanic bulla was opened on one side and the tympanic plexus was cut This was done to evaluate the proportion of sympathetic fibres passing to the inner ear via the tympanic plexus Unilateral cervical sympathectomy was performed in 2 animals Operations were performed about 3 weeks before sacrificing animals were decapitated under Nembutal® anesthesia and the cochleas were dissected out as quickly as possible They were freeze-dried and then treated with formaldehyde gas according to the histochemical fluorescence method of Falck & Hillarp (1962), (for details see Falck & Owman, 1965 and Corrodi & Jonsson 1967) Instead of embedding in paraffin, the specimens were infiltrated *in vacuo* for about 15 minutes in Epon, preheated to 60°C The epoxy resine were hardened and serial sections of segments of the cochlea were cut on a Jung microtome with glass knives

The sections were generally 6 to 8 μm thick and mounted in Entellan® A Zeiss fluorescence microscope with a dark field condenser was used In this microscope, fluorescent products of catecholamines appear green to yellow green in colour and those from 5 HT are yellow Photomicrographs were taken using Scopix G film

Electron microscopy

Six rabbits were pretreated with 5-OH DA (3,4,5-trihydroxyphenylethylamine hydrochloride) 4 × 20 mg/kg i v over a period of 48 hours (Tranzer & Thoenen, 1967)

re section ng

one an mal unilateral sectioning of the ypat c plexus was performed After 3 weeks an mal was pretreated with 5 OH DA and ri ced

Jnilateral sympathectomy was performed in nmals One of these had a survival time of 3 eks and was pretreated with 5 OH DA while second an mal was sacrificed after 8 days and s not pretreated with 5 OH DA

The an mals were sacrificed by decapitation der Nembutal[8] anesthesia The cochleas were d in on of two fixatives

(a) 3% glutaraldehyde (0 133 M sodium phos ate buffer pH 7 3) followed by 1% osmium rox de buffered in veronal acetate (Rhodin 54) for 2 hours

(b) Ice-cold 3% potassium permanganate in I M sod um phosphate buffer at pH 7 0 for minutes (Richardson 1966 Hokfelt 1968 69 Hokfelt & Jonsson 1968) After rinsing R ner solution the specimens were contrast ned en bloc in ice-cold 1% uranyl acetate in n er solution for 60 minutes

Th f xed spec mens were dehydrated in hanol and embedded in Epon (Luft 1961) ltrath n sections were cut with glass or dia ond kn ves on a LKB ultrotome Diamond ves were preferred because the bony spiralis sea wh ch enclosed the radiating nerve fibres as includ d in the specimens Sections from ecimens f xed in potass um permanganate were ntrast sta ned only briefly in lead citrate Reynolds 1963) while osmium fixed sections r contrast sta ned in uranyl acetate (Watson 58) and lead citrate The sections were exam d in a Siemens Elmiscop I electron microscope

RESULTS

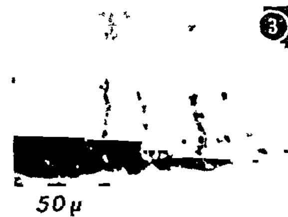
uorescence microscopy

l catecholamine-containing nerve fibres fluo ce green an indication of the presence of radrenal n In the cochlea no cell bodies were n to conta n biogenic amines

In the *mod olus* there was a moderate number adr nergic nerve fibres mostly coursing along







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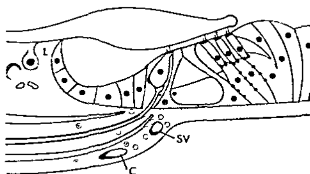


Fig 4 Schematic diagram illustrating the adrenergic innervation in the rabbit cochlea *SV*, spiral vessel of the tympanic lip, *C*, radiating capillary, *L*, limbus, O, adrenergic nerve terminal

the longitudinal axis of the modiolus There seemed to be a moderate, continuous, adrenergic perivascular innervation The plexus cochlearis (Balogh & Koburg, 1965) was included in some sections, but no concentration of adrenergic fibres was seen in this region of the modiolus fibres did not seem to have any relationship to blood vessels

In the *spiral ganglion*, adrenergic nerve fibres were mostly seen to pass through the ganglion, sometimes together with blood vessels Only occasionally were adrenergic nerve fibres seen to lie close to or partly encircle a ganglion cell with shining varicosities close to the cell wall (Fig 1)

The ganglion cells frequently contained yellow-red autofluorescent particles in the cytoplasm

In the rabbit the *osseous laminae* around the radiating nerve fibres were thick until they ended at the level of the habenula openings In stretch preparations it was therefore difficult to distinctly identify and separate adrenergic nerve fibres

In plastic sections, adrenergic nerve fibres were seen to run along the radiating nerve bundles, mostly as separate nerve fibres with varicosities It was difficult to ascertain whether adrenergic nerve fibres followed blood vessels or ran independently

In the *habenula region*, however one could often distinguish blood vessel innervation and innervation independent of blood vessels Blood vessel independent adrenergic nerve fibres seen close to radiating nerve bundles, concentration of varicosities just before habenula openings (Fig 2) An intense cence was also seen in association with the vessel of the tympanic lip (Fig 3) cochlear turns were investigated that the most extensive innervation habenula region existed in the second turn the spiral vessel seemed to be broad, intense shining bands and single cosities could not be resolved In sections from the *limbus region* a adrenergic innervation was seen Some fibres were seen to follow blood more often nerve fibres encircled cell an autofluorescent material

In the organ of Corti and stria catecholamine fluorescence was detected in stretch preparations or in sections.

After unilateral cervical fluorescence disappeared in the cochl operated side

Sectioning of the tympanic plexus influence the innervation pattern in

After administration of reserpine all cholamine fluorescence disappeared

Electron microscopy

After administration of the false transmitter 5-OH-DA, adrenergic nerve terminals easily recognized and differentiated from ones Adrenergic synaptic vesicles contain a precipitate consisting of the reaction product between the amine complex including 5-OH DA and the fixative (Tranzer & Thoenen 19 Hökfelt & Jonsson, 1968, Hokfelt, 19 Potassium permanganate fixed terminals recognized by the fact that these vesicles contained a dense core The vesicles were about Å in diameter but some were larger 1 000 Å Mitochondria were generally present the terminals

The cochlea had a very rich adrenergic innervation, with a concentration to the habenula region With the electron microscopic techniques used it was possible to distinguish clearly



5 Electron microscopic section from the cochlea [...]re a radiating capillary (*RC*) joins the spiral vessel [...] the tympanic lip (*SV*). Groups of adrenergic nerve terminals (*NT*) are seen close to the blood vessel *B*, bone, *R*, red blood corpuscle. Fixation in potassium permanganate ×12 600



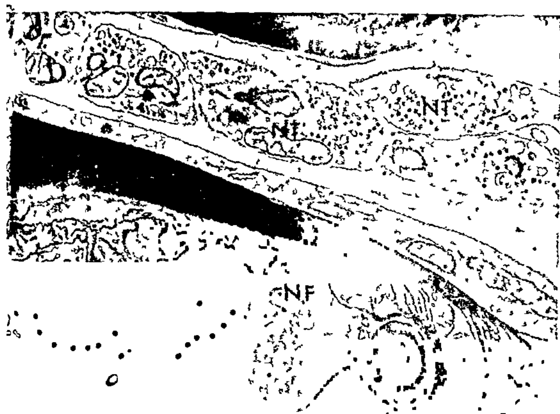
Fg 6 Part of the sp ral vessel of the tympan c l p (*SV*) and groups of adrenerg c nerve term nals (*NT*) Fixation in potass um permanganate × 30 750

innervation associated with *blood vessels afferent nerve fibres* and *free term nals* in the connective tissue

Blood vessel innervation

In the rabbit the outer spiral vessel was absent The spiral vessel of the tympanic lip (Fig 4) was built up of capı osseus spiral lam cades they sorr vessel in T junc tympanic lip region and the elec

travers



7 Electron microscopic section from the habenula ... on A row of adrenergic nerve terminals (*NT*) along a myelinated nerve fibre (*NF*) where it loses its myelin sheaths Fixation in potassium permanganate ×24 300

n The arterial blood vessels in the spiral ...ina and the tympanic lip were of capillary ... They were built up of endothelial cells and ...cytes but lacked a surrounding layer of ...ooth muscle cells

...n sections from the spiral lamina adrenergic ...ve terminals were found either singly or in ...ups along the capillaries The terminals were ... or partly surrounded by the Schwann cell ...ere the radiating capillaries joined the spiral ...sel one or two distinct groups of adrenergic ...ve terminals were regularly found and in ...e sections each group might contain up to ... terminals These groups of terminals did not ...m to be surrounded by a Schwann cell ...stly they were free and in other places the ...ole group was partly enfolded by a distended ... protrusion and it was not possible to decide ... originated from a Schwann cell or a fibro...st The terminals faced the endothelial cells of the blood vessel (Fig 5) The bony lamellae of the spiral lamina could extend beyond the spiral vessel which was sometimes enclosed by bone in three directions Adrenergic nerve fibres had a typical appearance with regular swellings i e terminals filled with vesicles Along the spiral vessel however this arrangement was partly concealed by many terminals assembled in elongated clusters In one ultrathin section as many as fifty terminals could be counted in a cluster Sometimes the spiral vessel was totally surrounded by terminals The existence of Schwann cells around the terminals was not a regular finding In some places a bundle of adrenergic axons might be surrounded by Schwann cells but mostly no Schwann cell was observed The nature of observed elongated cell protrusions was not possible to decide (Fig 6)

In the apical part of the cochlea the blood vessel innervation was less pronounced Single

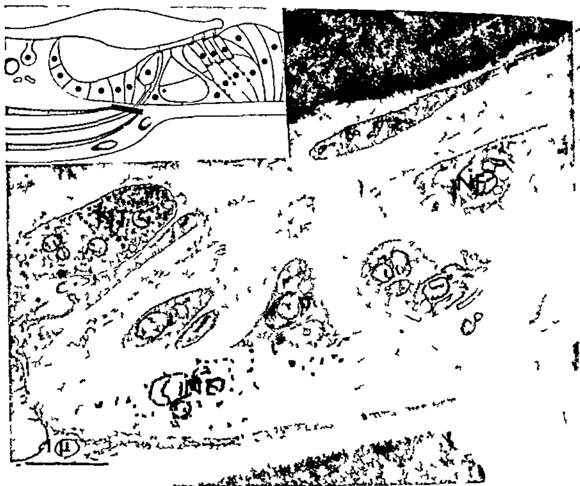


Fig 8 Electron microscopic section from the habenula region illustrating the relationship between nerve fibres (*NF*) after they have lost their myelin sheaths and an adrenergic nerve terminal (*NT*) Fixation in potassium permanganate ×20 000

terminals were seen along radiating capillaries in the spiral lamina but the pronounced innervation was not seen where these capillaries joined the spiral vessel Along the spiral vessel there were rows of terminals partly covered by a Schwann cell

Afferent nerve fibre associated innervation

Apart from the blood vessel innervation there was also an innervation associated with afferent nerve fibres Along radiating afferent nerve bundles and between myelinated nerve fibres rows of adrenergic nerve terminals were found The number of terminals increased towards the habenula region where terminals were located in rows or in groups (Fig 7) Groups of terminals were frequently seen around the afferent nerve bundles where the axons lose their myelin sheaths and turn upwards In horizontal sections adrenergic nerve terminals were seen around the nerve bundles after they lost their myelin sheaths in the habenula (Fig 8) The nerve bundles were surrounded by flat fibroblasts and the adrenergic nerve terminal always seemed to be located outside these cells It was observed that adrenergic terminals and also groups of terminals mostly were devoid of a Schwann cell

Free terminals

In the connective tissue towards the scala tympani and scala vestibuli terminals were

nd without any connection to blood vessels nerve fibres These nerve terminals were erally devoid of a Schwann cell cover. minals were more abundant in the tissue ards the scala tympani, especially in the hbourhood of the spiral vessel

re sectioning

in the tympanic plexus was resected there no definite change in the chochlea with rd to the amount or arrangement of nerve inals

fter cervical sympathectomy all adrenergic e terminals disappeared completely After eks the area around the spiral vessel of the panic lip, where the innervation was most ent, appeared empty Even after 8 days the inals were degenerated and it was not pos- to identify any remaining terminals

o adrenergic innervation existed distal to habenula region, in the organ of Corti or in stria vascularis

DISCUSSION

Falck Hillarp histochemical fluorescence hod is highly specific for the demonstration ertain biogenic amines (Falck et al, 1962, rodi & Jonsson, 1967) This method has er been used to show the presence of adrenaline-containing nerve fibres in cat, ea pig and rabbit cochlea (Spoendlin & tensteiger, 1966, 1967, Terayama et al, 5 1966, Vinnikov et al, 1966) In the ent study plastic embedding was used in erence to stretch preparation or cryostat sec ing, because in this way it is possible to t parts of the cochlea and section them at ible thicknesses and angles Fluorescent e fibres were not masked by the yellow auto- rescence of overlying bony lamellae Thus ontinuous adrenergic innervation was found distinct varicosities along nerve bundles o the level of the habenula openings As in cat and guinea pig (Spoendlin & Lichten- er, 1966, 1967, Terayama et al, 1965, 1966), as noted that there is a concentration of adrenergic innervation to the habenula region, encircling myelinated nerve bundles where the axons lose their myelin sheaths The most intense fluorescence, however, was seen around the spiral vessel of the tympanic lip, under the habenula, where thick bands of catecholamine fluorescence were seen

There was a close correlation between fluorescence and electron microscopic findings Administration of the false transmitter 5 OH DA makes it possible to use the electron microscope to identify and exactly localize biogenic amines in central, as well as in peripheral, neurons The existence of a blood–brain barrier prevents systematic administration of 5 OH DA when cen tral monoamines are investigated However, in the inner ear the adrenergic innervation emanates from the ipsilateral superior cervical ganglion and systemic administration of the false transmitter does lead to an accumulation in adrenergic synaptic vesicles

Fixation in potassium permanganate (Richardson, 1966, Hökfelt, 1968, Hökfelt & Jonsson, 1968) was preferred to glutaraldehyde and osmium tetroxide (Tranzer & Thoenen, 1967), for the purpose of illustrating the ultrastructure of adrenergic nerve terminals The existence of noradrenalin in small dense-cored vesicles, located in sympathetic nerve endings is well documented (De Robertis & Pellegrino de Iraldi, 1961, Richardson, 1962, 1966, Hökfelt, 1968, Hökfelt & Jonsson, 1968) Accumulation of biogenic monoamines and the false transmitters in adrenergic nerve terminals was first demonstrated in electron microscopic studies by Tranzer & Thoenen (1967) Using stretch preparations, Spoendlin & Lichtensteiger (1966, 1967) suggested a blood vessel innervation in the modiolus as well as an innervation independent of blood vessels in the periphery, giving rise to a nerve-associated habenula plexus Terayama et al (1965, 1966), using cryostat sectioning, claimed only a pure blood vessel innervation In an electron microscopic study Terayama et al (1968) failed to confirm their fluorescence microscopic finding that the spiral vessel under the inner pillar and tunnel of Corti had an adrenergic

innervation However, the electron microscopic results obtained after administration of a false transmitter (Densert & Flock, 1974) did show a continuous blood vessel innervation of the spiral vessel of the tympanic lip and a nerve fibre associated plexus in the habenula region In the rabbit this innervation is much more pronounced, with large groups or clusters of terminals where only single nerve terminals were found in the cat In rabbit it was observed that most terminals around blood vessels, afferent nerve fibres, and freely in the connective tissue, were not enfolded by a Schwann cell

In agreement with the results of Spoendlin & Lichtensteiger (1966, 1967) and Terayama et al (1965, 1966) no catecholamine fluorescence was found in the organ of Corti or stria vascularis Guinea pigs frequently have an outer spiral vessel, under the tunnel of Corti Using light microscopy Hawkins (1968) found an autonomic innervation of this vessel and Terayama et al (1967) found fluorescence at the site It should be noted that the outer spiral vessel is absent in the upper turns of the cat cochlea (Smith, 1954) and it is entirely absent in the rabbit cochlea

The possible functional significance of the adrenergic innervation in the cochlea has to be considered It might influence (*a*) blood vessels, (*b*) hair cell metabolism, (*c*) afferent and efferent axons

Different areas of the cochlea are involved in ion exchange and supply of oxygen and nutrients The relationship between the organ of Corti and the stria vascularis is not quite clear (Kimura, 1973) Experiments on cochlear blood flow have shown that obstruction of the radial vessels from the modiolus causes a loss of hair cells in the corresponding region of the organ of Corti (Alford et al, 1965, Lawrence, 1966) This might indicate that the oxygen and nutrient supply of the hair cells comes principally from the spiral vessels (Kimura, 1973) These capillaries are composed of endothelial cells and pericytes and are devoid of contractile elements The question arises why these vessels should be supplied with such a heavy adrenergic innervation Apart from a scanty innervation of capillaries in the limbus, other capillary areas in the cochlea lack an adrenergic innervation. Perlm[illegible] & Kimura (1955) observed that sympa[illegible] stimulation did not seem to influence the blo[illegible] flow in the *stria vascularis* Noradrenalin [illegible] influence the permeability of the spiral ves[illegible] However, there is a very large numbe[illegible] terminals in this area and it does not se[illegible] reasonable to believe that influence on perm[illegible] bility is the only physiological function of t[illegible] heavy innervation One possibility, that must [illegible] considered, is that catecholamines may have [illegible] direct influence on the hair cell metabolism [illegible] complex relationship exists between noradre[illegible] lin, cyclic AMP, and ions such as Ca^{++} and [illegible] The mode of action of noradrenalin depends [illegible] whether the receptors are of α or β type and [illegible] the inner ear this is unknown

Only a few investigations have been carr[illegible] out on the sympathetic influence on coch[illegible] microphonics and the results are contradictor[illegible] Beickert et al (1956) reported that the symp[illegible] thetic influence on chochlear microphonics di[illegible] appeared when the tendons of the middle ea[illegible] muscles were cut Seymour & Tappin (19[illegible] observed an initial small rise in cochlear m[illegible] phonics, followed by a decrease in ampl[illegible]

Except for a possible direct *effect* on ha[illegible] cells, there is morphological evidence fo[illegible] sympathetic influence even in the haben[illegible] region, where afferent axons have lost the[illegible] myelin sheaths Just below the basilar membra[illegible] the nerve bundles are surrounded by adrene[illegible] nerve terminals and prominent groups o[illegible] terminals are regularly found around the ne[illegible] bundles This is the site where the action pote[illegible] tials are supposed to be generated Noradren[illegible] released in this area might influence the th[illegible] hold for nerve impulse initiation

ACKNOWLEDGEMENTS

The author is deeply indepted to Ass Prof Åke Fl[illegible] and Prof Jan Wersäll for their support and g[illegible] The author wishes to express his appreciation to [illegible] Marie Louise Spångberg and Mrs Britta Flock for [illegible] technical assistance and especially to Mrs Britta Flock [illegible] artistic drawings

ZUSAMMENFASSUNG

: Verteilung der adrenergen Nervenfasern in der nnchencochlea wurde mit fluoreszenz- und elektronenmikroskopischen Methoden untersucht Das Interesse t vor allem der Habenularegion Nach Verabreichung es falschen Transmitters wurde die Feinstruktur der energen Nervenfasern und ihre Beziehung zu anderen ukturen studiert.

Ine große Anzahl von adrenergen Nervenendigungen rde rings um die strahlenformig sich ausbreitenden nenfasern nach Verlust ihrer Myelinscheiden beob-:et. Neben dieser Nervenfaserinnervation fand sich h eine ausgedehnte Innervation des spiralen Blutge es des Labium tympanicum unter den inneren Haar-en Dieses Blutgefaß von der Große einer Kapillare l kontraktile Elemente vermissen Im Corti'schen gan und in der Stria vascularis wurde keine adrenerge ervation gefunden Die adrenerge Innervation der chlea hat ihren Ursprung in dem ipsilateralen Ganglion rvicale sup Die Moglichkeiten eines adrenergen Ein sses auf die Schallperzeption werden diskutiert

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THE ACOUSTIC IMPEDANCE CHANGE AS A MEASURE OF STAPEDIUS MUSCLE ACTIVITY IN MAN

A Methodological Study with Electromyography

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ract The impedance change of the ear in response ound, widely used in clinical diagnosis, is generally rded to be caused by contraction of the stapedius le In the present study acoustic impedance change 00 Hz and EMG of the stapedius muscle were com d in subjects with unilateral ear drum perforation per cent of the maximum impedance change was hed at a sound level 5–6 dB above the one that gave of the maximum integrated EMG amplitude Impance change and integrated EMG were both approxtely linear functions of stimulus sound level at least to 20 dB above reflex threshold It was concluded the impedance change gives an adequate measure of stapedius muscle activity both at threshold and supra eshold levels The value of suprathreshold impedance nge measurements in clinical diagnosis was pointed t.

easurement of changes in the ear's acoustic pedance in response to sound stimulation has come an important diagnostic tool (Metz, 952, Klockhoff, 1961, Anderson, 1969, Peterson & Lidén, 1972) Such impedance changes re as a rule caused only by the contraction of e stapedius muscle The other muscle of the iddle ear, the tensor tympani muscle, is actiated in man only as part of a general startleeaction or in a few cases as an acoustic reflex Lidén et al, 1970) Its threshold is in any case onsiderably above the stapedius reflex threshold

This study was supported by Grants from the Swedish Medical Research Council the Medical Faculty, University of Umeå, P Frenckners fond, B Fromms fond and Karolinska Institutets fonder

The electromyographic activity upon contralateral sound stimulation has been recorded from the stapedius muscle in man through a perforation of the ear drum (Perlman & Case, 1939) and during operations in the middle ear under local anesthesia by Fisch & v Schulthess (1963) and Djupesland (1965) These studies mainly concerned the latency of stapedius reflex but Fisch & v Schulthess (1963) also studied the reflex threshold and observed that the EMG activity was dependent on the stimulus sound intensity In none of these studies was the EMG activity correlated to the change in acoustic impedance

In experiments on lightly anesthetized rabbits it has been shown (Borg, 1972) by the bilateral recording method developed by Møller (1961) that changes in the ear's acoustic impedance are closely correlated to the threshold for EMG activity of the stapedius muscle Furthermore, good correlation was found between the amplitude of the integrated EMG and the change in impedance

There is very incomplete knowledge of the correlation in man between the change in acoustic impedance during sound stimulation and the activity of the stapedius muscle as measured more directly Jepsen (1955) compared threshold for impedance change with threshold for directly observed movement of the stapedius tendon upon contralateral sound stimulation in two

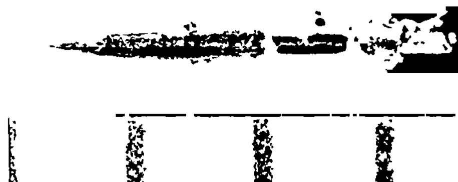


Fig 1 Tungsten electrod and milli metre scale

patients with unilateral perforation of the ear drum The threshold determined by direct observation was less than 10 dB higher than the threshold for impedance change

The aim of the present work was to determine the correlation between the EMG activity of the stapedius muscle and the acoustic impedance change with respect to threshold sensitivity and suprathreshold response amplitude This was accomplished by simultaneous recordings of ipsilateral impedance change in one ear and EMG in the other ear in patients with unilateral ear drum perforation The results showed a good correlation between recordings obtained with the two methods

MATERIAL AND METHODS

The experiments were performed on a total of 37 subjects Thirty two of these comprised a control group previously described by Borg & Zakrisson (1974) Selected from young university students, they were used to determine the normal difference in sensitivity between the ipsilateral and contralateral stapedius reflex Five subjects, the Experimental Group, had an ear drum perforation with visible stapes and stapedius muscle tendon in one ear In this ear they had hearing thresholds of 20–75 dB HL (Hearing Level) according to ISO Standard (1964) in the frequency range 0 125–2 0 kHz In the other ear they had hearing thresholds within 15 dB according to ISO Standard (1964) at the frequencies used to elicit the stapedius refle Four of the subjects had normal ear drum this ear according to otomicroscopic and the fifth an ear drum scar The Experiment Group was used for EMG recordings

Sound stimulation The reflex activity, both EMG and impedance change, was obtained response to bursts of pure tone at 0 5 or 2 0 k (rise-time to 90% and fall time to 10% were or 2 msec) of 1 sec duration The stimuli were increased in 4 dB steps from below reflex thresh old to about 130 dB SPL (sound pressure lev and then lowered again to below reflex thresh old Two stimulus presentations were thus made at each sound level

Impedance change was measured with the method developed by Møller (1960, 1961) This technique allows a simultaneous recording of ipsi- and contra lateral impedance change at 800 Hz The 800 Hz signal representing the chang of impedance during stapedius contraction was recorded on a two channel tape recorder (Revo A 77) During playback the signal was rectifie and low-pass filtered (bandwidth 32 Hz, 18 d per octave) The amplitude of the impedanc change signal was measured at the end of th stimulus and expressed in per cent of the maxi mum response obtained at each session The value was plotted as a function of stimulus inte sity to produce stimulus–response curves. In th control group bilateral recordings of impedanc change were made In 4 of the patients of th Experimental Group impedance change was re

orded simultaneously with the EMG In the fifth patient no impedance change was obtained which could be due to middle ear pathology related to the ear drum scar (Klockhoff, 1961)

EMG recordings were made with tungsten microelectrodes (Fig 1) of the type described by Vallbo & Hagbarth (1968) The electrodes were coated with Araldite except for 0 4 mm at the tip Their length was about 3 mm The tip diameter was between 1 and 10 μm and the impedance was about 40 kOhms at 1 0 kHz

The recording electrode was introduced through the perforation of the ear drum and inserted into the tendon of the stapedius muscle It was oriented as near the muscle as possible, parallel to the tendon No local anesthesia or sedation of the patient was utilized The reference electrode was a similar, but longer tungsten needle having a greater uncoated area It was inserted in the ipsilateral ear lobe The EMG signal picked up by the electrode was amplified by a Tektronix 2A61 amplifier with an input impedance of 2×10 MOhms The frequency response was between 60 Hz and 6 kHz which gave the best signal to-noise ratio in these recordings The amplified signals were displayed on a Tektronix 564 oscilloscope and monitored on a loud speaker The activity was recorded on one channel of the tape recorder, the impedance change of the other ear being simultaneously recorded on the other channel The integrated EMG responses were obtained by low-pass filtering the rectified EMG signal Active low-pass filter of third degree, i e attenuation rate of 18 dB per octave and cut off frequency of 39 Hz (3 dB) was used The amplitude of the integrated EMG signal at the end of the stimulus tone was measured and expressed in percentage of the maximum response amplitude obtained in each session These relative values were plotted as a function of stimulus intensity to yield stimulus–response curves Such curves were found to be a reproducible measure of the EMG activity of the stapedius muscle Fig 2 shows two stimulus–response curves based on integrated electromyograms obtained in one subject with about 20 min interval during the same

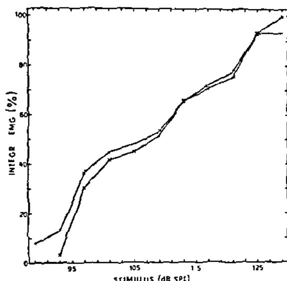


Fig 2 Two stimulus response curves for integrated EMG . . . perimental session Each value is the average of two measurements Contralateral stimulation with pure tone of 0 5 kHz

experimental session It is seen that the curves correlate closely

The average of stimulus–response curve was calculated in the following way The sound intensity giving 50 % of maximum amplitude for each curve was chosen as reference and given the value zero dB For each 10 % of amplitude the corresponding intensity was determined and expressed in relation to the value giving 50 % Thus for each stimulus–response curve 10 dB values were obtained corresponding to each 10 % amplitude from 10 to 100 % The average dB-value for each 10 % amplitude was then calculated for the group of curves being considered, thus defining the shape of the average curve The position of the curve was obtained as the average sound pressure level corresponding to the 50 % response amplitude for the sample of curves

In the control group the difference between ipsilateral and contralateral stimulus–response curves was calculated for each subject The individual differences in intensity giving 10, 20, 30 % etc up to 90 % of the maximum response for each curve were determined and ... age value was calcu ... 10 % ... de

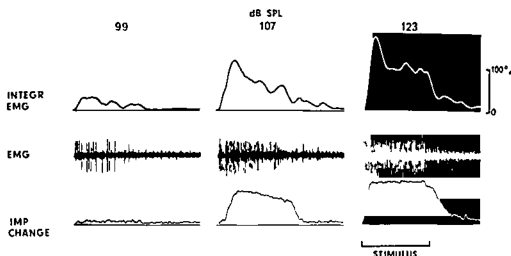


Fig 3 Simultaneous recordings of EMG of the stapedius muscle of the left ear (middle row) and impedance change in the right ear (bottom row) in response to 2 0 kHz pure tone stimulation of one second duration in right ear Integrated EMG (rectified and low pass filtered at 3 9 Hz) is illustrated in the upper row Vertical scale shows integrated EMG and impedance change amplitude in per cent of maximum obtained value as measured at the end of the stimulus One subject

RESULTS

A *General characteristics*

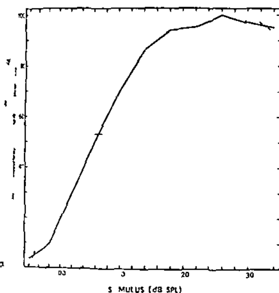
Simultaneous recordings of EMG of the stapedius muscle of one ear and change of the acoustic impedance of the other ear were made Fig 3 shows the course of the stapedius activity elicited by a 2 0 kHz pure tone at three different intensity levels in one subject The upper row shows the integrated (rectified and low pass filtered) EMG, the middle row the EMG signal, and the bottom row the change of the acoustic impedance at 800 Hz At the lowest stimulus intensity (99 dB SPL) the EMG activity seen probably represents only one motor unit A small impedance change is also seen The firing frequency of the motor unit is initially high, but declines after about 300 msec At the higher intensity levels shown in Fig 3 several motor units are activated and the impedance change is more prominent

Especially at the highest intensity illustrated in Fig 3 the EMG and the integrated EMG as well exhibit an initial peak which soon takes on a lower, fairly stable level In the impedance change on the other hand there is no or only a very slight tendency to an initial peak After the end of the stimulus there is an afterdischarge in the EMG during about 400 msec at the two highest stimulus intensity levels Fig 3 also shows the slow return of the impedance change to zero after the end of the stimulus corresponding to the afterdischarge of the EMG

The latency from the start of the stimulus to the first motor unit potential in general decreased when the stimulus level was raised The lowest value obtained was 12 msec (stimulation with 2 0 kHz pure tone at 124 dB SPL, 2 msec rise time) Impedance change showed a onset, making determinations of latency very uncertain The results thus show that the stapedius reflex has properties that can be expected from a polysynaptic reflex (cf Borg 1973)

B *Threshold sensitivity*

The threshold for EMG activity can be defined as the lowest stimulus sound level giving motor unit potentials It is more difficult however to determine the sound level giving the smallest impedance change The threshold sensitivity for the two measuring techniques has therefore been compared in the present study on the basis of those sound intensity levels needed to obtain responses equal to 10% of the maximum impedance change and integrated EMG respectively (Moller, 1962) In the 4 subjects in whom



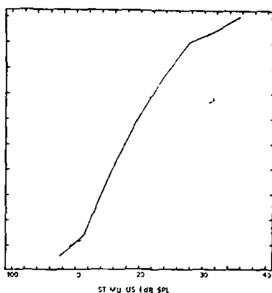


Fig 4 Stimulus response curves for ipsilateral impedance change (——) and for contralateral integrated EMG (- - -) Each graph shows results from one subject The amplitudes of the integrated EMG and of the impedance change were measured at the end of the stimulus and expressed in per cent of the corresponding maximum obtained amplitude Stimulus was 0 5 kHz pure tone of 1 sec duration

individual comparisons could be made the 10% level of the integrated EMG was reached at a stimulus sound level (0 5 kHz) which was on the average 1 2 dB below the 10% level of the impedance change This value represents the difference between the ipsilateral impedance change and the contralateral EMG activity In the control group of 32 subjects the threshold (10% level) measured as impedance change was 3 5 dB lower for ipsilateral than for contralateral stimulation with 0 5 kHz pure tone Ten per cent of the maximum amplitude of the integrated EMG was thus reached at a sound level 5-6 dB below the level giving 10% of maximum impedance change at 800 Hz Motor unit activity could be recorded on average at another 2 dB lower sound level The utilized threshold value for impedance change 10% of maximum amplitude was thus reached at a sound intensity 7 8 dB above the level giving the first motor unit activity of the stapedius muscle

C *Stimulus-response relations*

The amplitudes of the integrated EMG and of the impedance change signal at the end of each stimulus were used as measures of the muscle activity Both were expressed in per cent of the corresponding maximum obtained response amplitude Fig 4 shows stimulus-response curves for the ipsilateral impedance change (continuous line) and contralateral integrated EMG (broken line) in 2 subjects based on such measurements It is seen that the amplitude relation between the EMG and the impedance change varied as a function of stimulus intensity The difference between the curves is in part due to the fact that the impedance change was obtained upon ipsilateral and the EMG upon contralateral sound stimulation It is also possible that the EMG recorded was not representative of the electrical activity of the whole stapedius muscle but mainly showed the activity of a relatively small region in the vicinity of the recording electrode

More reliable information on the relation of the total EMG activity of the stapedius muscle and the impedance change ought to be gained from average stimulus response curves based on several subjects Fig 5 shows such average curves obtained in the Experimental Group The broken line illustrates the EMG upon contralateral stimulation The thin continuous line rep-

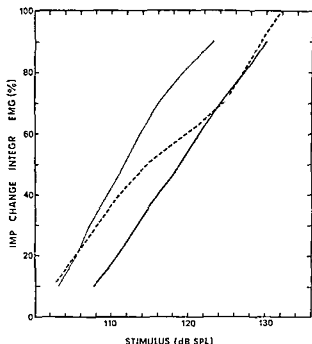


Fig 5 ------, average of the stimulus–response curves for contralateral EMG in the Experimental Group ———, average of stimulus-response curves for ipsilateral impedance change in 4 of the subjects of the Experimental Group ———, average contralateral stimulus-response curves for impedance change as based on the ipsi lateral recordings of the 4 subjects of the Experimental Group corrected for the average difference between ipsilateral and contralateral reflex in the control group Stimulus was pure tone at 0 5 kHz of 1 sec duration

resents the impedance change upon ipsilateral stimulation The heavy continuous line is obtained from the thin line by correcting for the average difference in sensitivity between the ipsilateral and the contralateral reflex in the control group Thus it represents an estimation of the impedance change of the "EMG ear" upon contralateral sound stimulation and can be directly compared with the broken line A more stable correlation between these average curves than between the individual curves of Fig 4 is evident The curves of Fig 5 are approximately rectilinear up to about 130 dB SPL, i e more than 20 dB above threshold The difference between contralateral EMG (broken line), and contralateral impedance change (heavy continuous line) is, at threshold as well as at suprathreshold levels, less than 6 dB

DISCUSSION

The results showed that the threshold o stapedius muscle reflex was 5–6 dB higher measured as change in acoustic impedan 800 Hz than when measured as integrated E The impedance change and the integrated were both approximately linear functio stimulus level up to at least 20 dB above threshold The average difference betwe two stimulus response-curves was at all less than 6 dB

The difference in threshold sensitivity tained with the two methods was small an regarded to be without clinical impor Possible explanations for the difference a the lowest degree of EMG activity w accompanied by any pull in the tendon, c the acoustic impedance at 800 Hz was fluenced by the weakest change of tensio latter alternative is supported by the fi by Borg (1968) He showed that the sta reflex influences sound at 0 5 kHz at a lo of muscle activity whereas sound at 1 4 is influenced only well above reflex thr Since the measuring tone used (800 H between these two frequencies, it might unaffected by very weak stapedius contra Impedance change measurements at a measuring tone frequency would thus p give a more sensitive measure of the sta activity at threshold Peterson & Liden determined the stapedius reflex threshol impedance change at different measurin frequencies, 220, 625 or 800 Hz The th for impedance change at 800 Hz was ob at a stimulus sound level 2 1 dB abo threshold for impedance change at 220 3 4 dB below the impedance change at 6 Since the three values are close to each c is likely that all three of the measurin frequencies give threshold values very n that for pull in the stapedius tendon

It would be [illegible] terest if the te developed by th directly [illegible]

In rabbits Wersall (1958) found that the tension was a linear function of stimulus sound level within a 30 dB range At higher levels there was a saturation

No tension measurements from the stapedius muscle are available in human studies There are however several investigations where the EMG has been correlated to the tension of extremity muscles Bigland & Lippold (1954) and Stephens & Taylor (1972) for example found a mainly linear relationship between the integrated surface EMG and the tension at isometric contraction The present results showed that the integrated EMG and the impedance change were both approximately linear functions of stimulus sound level It thus seems justified to conclude that the impedance change is largely proportional to the tension developed by the stapedius muscle

Clinical aspects

The threshold of the stapedius reflex measured as change in the ear s acoustic impedance is commonly used in clinical diagnosis It is evidently close to the threshold for contraction of the muscle In a few clinical studies also the stimulus response curves of the stapedius reflex have been given attention (Klockhoff, 1961, Djupesland et al, 1967, Peterson & Liden, 1972) Suprathreshold stapedius activity has been used for diagnostic purpose by Burghoff (1968) Anderson et al (1969), Blom & Zakrisson (1974) Anderson et al (1969) showed that patients with retrocochlear affections exhibited an abnormally rapid decay of the impedance change during constant low frequency pure tone stimulation

The present results showed that suprathreshold impedance changes and stimulus–response curves are also good measures of the activity of the stapedius muscle They can thus probably be used to reveal disturbances in the function of the cochlea, the retrocochlear reflex connections or in the stapedius muscle This is supported by findings in animal experiments (Borg 1973) where chronic lesions in the brain ... the stimulus–response curve of the stapedius reflex Furthermore, comparisons between ipsilateral and contralateral stimulus–response curves might increase the diagnostic possibilities in disturbances of the lower auditory system

ZUSAMMENFASSUNG

Die Impedanzanderung des Ohres bei Beschallung wird in der klinischen Diagnostik weitgehend angewandt und als ihre Ursache wird nunmehr allgemein die Kontraktion des Stapediusmuskels angesehen Um nahere Daten uber die Korrelation zwischen der akustischen Impedanzanderung bei 800 Hz und dem Elektromyogram (EMG) des Stapediusmuskels zu erhalten wurden in der vorliegenden Arbeit Untersuchungen an Patienten mit einseitiger Trommelfellperforation durchgefuhrt 10 % der maximalen Impedanzänderung wurden bei einem Schallpegel von 5 bis 6 dB uber dem Pegel erhalten bei dem die Amplitude des integrierten EMG s 10 % der Maximalamplitude des integrierten EMG s war Die Impedanzanderung und das integrierte EMG waren beide annäherungsweise lineare Funktionen des Schallpegels bis zu Intensitäten von mindest 20 dB uber der Reflexschwelle Es ist daraus zu schliessen dass die Bestimmung der Impedanzänderung ein adäquates Mass der Stapediusmuskelaktivitat sowohl bei Schwellenpegeln als auch bei uberschwelligen Pegeln ergibt Abschliessend wird auf den Wert derartiger Messungen uberschwelliger Impedanzanderungen in der klinischen Diagnostik hingewiesen

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FOCUS LOCALIZATION IN TWO CLINICAL TYPES OF OTOSCLEROSIS

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stract In the present study middle ear pathology was -vestigated in two clinical types of otosclerosis No -fference in localization of otosclerotic foci in the iddle ear has been found between these clinical types e tympanical the other cochlear Superior and inferior arginal footpl te

u bicrural involvement is predominantly found thick complete footplate lesions A progressive type otosclerosis can be found in male adolescents This pe shows complete thick footplate invasion without erceptive loss Postoperative hearing gain was satis ctory and to the same degree in both types of clinical tosclerosis regardless the type of middle ear pathology xcept for round window otosclerosis

Much has been written about the histopathology of otosclerosis, by far the most of which was in qualitative descriptive way Quantitative state ments on the frequency and distribution of oto- sclerotic foci in the bony capsule of the labyrinth have been published by Guild (1944) Nylen (1949) and by Ruedi & Spoendlin (1957)

A correlation between the type of footplate pathology and the type of hearing loss in oto- sclerosis has initially been assumed by Farrior (1958) but in his later work (Farrior, 1963) he has found no significant correlation between the degree of deafness and the severety of the foot- plate pathology This in is agreement with others (Antoli–Candela 1960, House, 1959) Ludman (1962) came to another conclusion It has been assumed by him that the thickness of the foot- plate lesion is related to the degree of deafness

Two clinical types of otosclerosis have been reported by us in a previous study (Marres et al , 1973) One clinical type, which is called type I otosclerosis, concerns the patients having a relatively moderate hearing loss for all sound frequencies The other clinical type, designated type II otosclerosis, comprises the patients hav- ing a more severe hearing loss and the poorest hearing at the highest frequencies It is assumed by us that type I otosclerosis is identical with the tympanical type of Politzer–Siebenmann and that type II otosclerosis is identical with the Lermoyez type of cochlear otosclerotic in- volvement

In the present report a comparison between the type of pathology in both clinical types of otosclerosis has been made in order to see whether pathology is correlated with type of hearing loss

MATERIAL AND METHOD

Middle ear pathology was recorded following the coding scheme by Farrior (1958) in a group of 271 patients with otosclerosis Hearing loss in both air and bone conduction at the frequen- cies 0 5, 1, 2, 4 and 8 kHz was measured shortly before stapedectomy and insertion of a Teflon piston prosthesis and also one year after the operation The coding system is given in Table I

All calculations were performed with the IBM System/370 model 155 computer of the ' Uni- versitair Reken Centrum" at Nijmegen The frequency of each pathology code was scored in both groups of patients The combined fre- quencies of pathology codes were a and tested in a contingency tabl

Table I *The frequencies of the various aspects of otosclerosis, coded according to Farrior (1958)*

Modifications codes VIII and 2 denote Farrior s codes VII and VIII respectively

Codes	Type I	Type II	Total
Anatomical situation			
Depth of the footplate			
A1 superficial	78	43	121
2 moderately superficial	76	30	106
3 moderately deep	21	12	33
Angulation of crura			
B1 upright	135	70	205
2 downward tilt (free)	35	14	49
3 downward tilt (fixed)	4	1	5
Pathology			
Footplate otosclerosis			
II1 none	1	0	1
2 anterior	34	12	46
3 posterior	23	9	32
4 superior marginal	42	18	60
5 inferior marginal	55	22	77
6 ¼ footplate	15	7	22
7 ½ footplate	18	9	27
8 ¾ footplate	13	5	18
9 complete footplate (thin)	42	24	66
10 complete footplate (thick)	32	21	53
Crural otosclerosis			
III1 none	119	55	174
2 anterior crus	24	10	34
3 posterior crus	14	7	21
4 bicrural	22	14	36
Round window otosclerosis			
IV1 none	107	46	153
2 marginal without closure	1	1	2
3 partial closure	0	2	2
Thickness of otosclerosis			
V1 very thin	91	42	133
2 thin	51	20	71
3 moderately thick	38	23	61
Colour of otosclerosis			
VI1 white	116	60	176
2 nearly white	48	19	67
3 pink	11	5	16
Miscellaneous pathology			
VII1 mucous membrane webs	6	5	11
2 fibrous adhesions	5	0	5

test) against the expected frequencies that could be calculated Trivial combinations which were the direct consequence of a certain excess in the coding system, were ignored A "significant combination" is defined as a combination, which incidence has been proved to be significantly higher than could be expected or could not be ascribed to chance alone other than in 5% of the cases

Special interrelations between pathology coding items have been sought The relevance of age and sex have been considered in connection with the type of hearing loss the type of middle ear pathology and the hearing gain experienced by the patient Miscellaneous pathology is presented and special attention is given to the patients with a very severe hearing loss

RESULT

Frequencies of otosclerotic foci

The frequency of each pathology code in the operation files is given in Table I for both type I and II patients There is no significant difference between the frequencies of the codes in type I and II patients, nor in males and females (not shown here) In view of this, most of the following is about all categories of patients, type I and II, males and females together

The incidence of total footplate otosclerosis (II9 and II10 in Farrior s coding system, Table I) is high, which indicates that we are dealing with extensive lesions Round window otosclerosis was very seldom seen (2.5% of the cases)

Combined frequencies of foci

A relevant part of the computer generated cross table is shown in Table II From the observations presented in this table which proved to be significant, the following conclusions can be drawn

1 In the deeper placed stapes footplates (A2, 3) more downward tilted crura (B2, 3) are found and more superior (II4) or inferior (II5) marginal footplate otosclerosis

2 Superior (II4) and inferior (II5) marginal footplate otosclerosis are significantly associated They are found in combination in about half of the cases

3 Few crural involvement, i e III1, is found in combination with marginal footplate otosclerosis (II4, 5) or in very thin (V1) otosclerosis

ble II *Combined frequencies*

	A2,3	B2,3	II2	3	4	5	10	III1	2	3	4	VI	VII
23	139												
23	43	54											
2			46										
3				32									
4	43				60								
5	54				42	77							
0							53						
1					50	63		174					
2			17						34				
3				12						21			
4							20				36		
1								107				133	
1							44						176

Anterior crus otosclerosis (III2) is associ-
d with anterior pole otosclerosis (II2) as is
terior crus otosclerosis (III3) with posterior
tplate otosclerosis (II3)

Bicrural involvement (III4) is predominant-
found in combination with thick complete
tplate lesions (II10)

Complete thick footplate otosclerosis (II10)
most instances has a white aspect (VI1)

tplate pathology

m the previous findings it can be deduced
t the most common types of footplate patho-
y are

Polar otosclerosis (26 % of the patients) at
anterior (II2) or the posterior (II3) pole of
footplate, sometimes at both poles (10 % of
cases) and in 1/3 of the cases involvement
the associated crus (III2 or III3 respectively)

Marginal otosclerosis (35 % of the patients)
the superior (II4) or at the inferior (II5) mar-
and in half of the cases at both margins of
footplate In marginal otosclerosis in most
tances (about 85 % of the cases) both crura
intact (III1)

Complete footplate otosclerosis (44 % of
patients) in 55 % of the cases thin (II9), in
other cases thick (II10) and in those cases
ferentially white (VI1, in 83 % of the cases) and in about 1/3 of the cases with bicrural involvement (III4)

Overlapping of type 1 and 2 pathology, mixed polar and marginal otosclerosis, was present in 7 % of the patients, extending to fully circumferential otosclerosis (II2, 3, 4, 5) in 1 %

In Table III the incidence of the various types of footplate pathology is shown for the different clinical types of otosclerosis From this table it can be seen that no significant difference exists between the type of footplate pathology in the two clinical types

Footplate pathology regarding clinical type, sex and age

The only significant difference in footplate pathology between the sexes lies in the combined frequency of superior marginal (II4) and inferior marginal (II5) otosclerosis This combination occurs almost twice as much in females (see Table IV) In fact the high incidence of this combination in females is responsible for the significance of this combination as mentioned before

Other types of footplate pathology are equally distributed with respect to the sexes and age classes, with only one important exception In a group of 12 boys, 14–19 years old, 7 had thick complete footplate otosclerosis (II10) and

Table III *The incidence of the various types of footplate pathology in the two clinical types of otosclerosis*

Footplate otosclerosis	Type I	Type II	Total
None	1	0	1
1 Polar	52	18	70
2 Marginal	65	30	95
3 Complete	74	45	119
1,2 mixed	13	6	19
Unspecified	4	1	5
No of patients	183	88	271

had type I hearing loss (the boy with type II hearing loss having round window otosclerosis with partial closure, i e IV3) The high incidence of type I hearing loss and thick complete footplate otosclerosis are very significant in this group of young males, as is the combined incidence of these two characteristics The young male group itself is a significant finding in the whole age distribution of the patients It might be a very progressive type of otosclerosis with a high incidence of thick footplate lesions, but without perceptive loss (i e type I hearing loss)

… result as related with clinical type, … of pathology, sex and age

As shown in Table V, there is no relation between prognosis and clinical type of otosclerosis, sex, type of footplate pathology or age Hearing gain is good in all categories and the average gain in the Fletcher-index one year after the operation is 32 dB In miscellaneous pathology hearing gain is identical This is not the case in round window otosclerosis, however Only one of the (four) patients with round window otosclerosis (having IV2, marginal without c… had sufficient hearing gain This patient was only one having round window otos… combined with type I hearing loss The … patients with round window otosclerosis (… IV2 and two IV3, partial closure) had … hearing loss and even for that type of … they had extremely severe hearing loss … patients had *hearing loss* (1) or poor gain (2) one year after the operation T… of these patients the shift in the Fletcher … *after the operation* was significantly small… compared with the more regular type II … Although the incidence of round window … sclerosis was too low for statistical testing, ro… window otosclerosis in type II hearing loss se… to be associated with severe perceptive inv… ment It seems to have a poor prognosis, because it can be associated with extensive … sclerosis with cochlear involvement … 1960)

Table IV *The combined frequencies of superior marginal (II4) and inferior marginal (II5) footplate otosclerosis in males and females*

Combinations	Males	Females	Total
Realized	12	30	42
Not realized	13	5	18
Maximum number, which could have been realized	25	35	60

Table V. *The mean hearing gain at the … frequencies in type I and II patients … females of various age classes, in a… of footplate pathology*

The mean hearing … [illegible]

ment (n …)

— = missing observations, figures in parentheses mean hearing gain calculated from less than 4 …

Age class (yrs)	Type I Footplate lesions						Type II					
	Complete						Complete					
	Thin		Thick		Minor		Thin		Thick		V…	
	m	f	m	f	m	f	m	f	m	f	m	f
0–24	33	27	29	31	33	29	—	—	—	—	—	
25–39	36	28	31	27	34	32	38	58	(24)	(38)	31	
40–75	31	32	—	29	26	32	39	38	35	32	30	

Average gain 32 dB

Footplate pathology and very severe hearing …

In the group of patients with a very severe he… ing loss (21 after elimination of 3 patients …

e VI *Survey of the literature on macroscopic footplate lesions in bony stapes ankylosis com-
on with present study*

incidence of	small lesions			moderate lesions					extensive lesions		
rence no	1	2	3	4	5	6	7	8	9	10	11
of cases with tes ankylosis	?	*several* hundred	49	1 937	125	78	7 000	28	35	102	271
of pathology (%)											
ible lesion	36	—	—		—	9	—	—	—		0 3
				8						*33*	
r footplate	41	73	68			63	58	25	29		19
g nal footplate			14			4			8		28
	14	20		75	70		20	75		16	
ed polar marginal			18			*5*			—		7
plete footplate	9	7	—	16	30	19	22	1	63	51	44

rences 1 *Bellucci 1958*, 2 Farrior, 1960, 3 Fleischer, 1958 4 Antoli Candela 1960 5 Farrior, 1963 6 García
hez & Iurato 1969 7 Kaplan & Shambaugh, 1961, 8 Rüedi & Spoendlin 1957 9 del Bo & Bergomi, 1970, 10
es & Jackson 1962 11 present study

nd window otosclerosis) half of the patients (3) above 60 years of age are present There no difference in footplate pathology between e 6 patients and the other 7 in the same age up, which had relatively moderate hearing s before the operation A normal hearing gain experienced by these 6 patients as well as all others in the group with very severe hear- *loss*

DISCUSSION

e of footplate pathology

: types of footplate pathology as reported in literature, which could be reclassified on the is of the present classification (see Table III) listed in Table VI Only the selected cases orted by del Bo & Bergomi (1970) and those Dawes & Jackson (1962) show a higher idence of extensive lesions than reported in present paper (see Table VI)

mbination and development of foci

asion of the nearby crus from a polar focus, st often anteriopolar, is a common finding toli Candela, 1960 Bellucci, 1958 Farrior, 8, 1960, 1963, García-Ibáñez & Iurato, 1969) n the present paper, though more posterior olvement is reported here The predominance bicrural involvement in the more affected footplate as presented here, has also been reported by Antoli-Candela (1960), Bellucci (1958) and by Farrior (1960) The tendency toward bicrural involvement in inferior marginal footplate otosclerosis as reported by Farrior (1960) could not be shown in the present study On the contrary, it is a significant finding that few crural involvement was seen in marginal otosclerosis It cannot be confirmed that a deeply placed footplate with downward tilted crura predisposes toward crural or complete otosclerosis (Farrior, 1958, 1960) Indeed, more downward tilted crura and more marginal (but not more extensive than that) otosclerosis were found in the deeper placed footplate but, as stated before, in fact few crural involvement in combination with this marginal otosclerosis was seen

The findings in early lesions as reported in the literature and the present findings suggest the following general "scheme of development" of the footplate lesions

A primary site of affection seems to be the anterior footplate pole, though other sites might be the posterior pole or one or both margins as well Extension of the primary lesions can take place in all directions, in many instances leading to crural involvement as soon as the nearby pol is involved

Types of pathology and types of hearing loss

The fact that no difference in middle ear pathology has been found between type I and type II otosclerosis implies that the difference between these types of otosclerosis must be sought outside the middle ear. It can be shown that besides middle ear pathology type I and II patients have one parameter in common: the airbone gap. In both groups of patients this parameter has the same value before the operation. Moreover, the reduction in air bone gap or hearing gain after the operation is exactly the same in both groups. Even in the type II patients with a very severe hearing loss before the operation, round window otosclerosis excluded. Apart from the stapes fixation with the concomitant conduction loss, type II otosclerosis probably has cochlear involvement leading to additional perceptive hearing loss.

ZUSAMMENFASSUNG

Bei Patienten mit zwei klinisch verschiedenen Arten der Otosklerose, bei denen Stapedektomie vorgenommen war, wurde die Pathologie des Mittelohres untersucht. Die Lokalisation der otosklerotischen Herde im Mittelohr zeigte bei beiden Typen — dem tympanischen oder cochlearen — keinen Unterschied. Bei der Fussplatten-otosklerose wurde besonders bei Frauen öfters eine Kombination von Herden am oberen und unteren Rand gefunden. Bei dieser Lokalisation wurden wenig krurale Formen beobachtet. Die Crura waren meistens durch Ausbreitung des Prozesses vom nachstgelegenen Pol aus betroffen. Beteiligung beider Crura am Krankheitsprozess wurde hauptsächlich bei totaler Fussplattenotosklerose gefunden. Bei mannlichen Adoleszenten zeigte sich ein progressiver Typ von Otosklerose, wobei eine dicke, total befallene Fussplatte ohne Perzeptionsverlust vorlag. Bei beiden klinischen und bei allen beschriebenen pathologischen Typen von Otosklerose war der postoperative Horgewinn gleich gut. Ausgenommen waren lediglich die Patienten mit Herden am runden Fenster.

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SCHÄDIGUNG DES INNENOHRES NACH TYMPANOPLASTIK TYP I

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(Eingegangen am 25 Januar 1974)

Kurzfassung Die Auswertung der Tonaudiogramme von 930 Tympanoplastiken Typ I, die in den Jahren 1960–1972 an der Freiburger Univ Hals-Nasen-Ohrenklinik operiert wurden, zeigt 3 Wochen nach der Operation eine Innenohrschädigung bei 17 %, 3 Monate nach dem Eingriff noch eine solche bei 10,8 % der Patienten Als Ursache dieser Innenohrstörung werden diskutiert

1 Die Traumatisierung des Innenohres während der Operation

2 Die Tatsache dass die Operation oft am kranken bzw krankgewesenen Ohr erfolgt

3 Die individuelle Empfindlichkeit des Ohres gegen Belastung.

Schon bald nach Entwicklung der Tympanoplastik wird über Einzelfälle einer Schädigung des Innenohres, dokumentiert durch ein postoperatives Absinken der Knochenleitungsschwellenkurve evtl bis zur Ertaubung berichtet (Beickert, 1958, 1962, Schwetz, 1957, 1958) Spätere Veröffentlichungen bestätigen diese Erfahrung (Bandtlow, 1960, Bartual, 1964, Heermann, 1960, Palva et al , 1973, Pfaltz et al , 1962, Proctor, 1964, Rentzsch, 1973, Wullstein, 1968), wobei besonders die Tympanoplastiken der Typen III und IV betroffen sind Daneben wird betont, dass auch bei Typ I eine Beeinträchtigung der Innenohrfunktion möglich ist (Ballantyne, 1970 Beickert, 1958, 1962, Bicknell, 1971, Ley, 1969, Thornburn, 1971) Eine Reaktion des Innenohres bei diesem einfachsten Typ der Tympanoplastik ist erstaunlich und nur schwer zu erklären Wird doch hier im Gegensatz zu den ausgedehnten Eingriffen der Typen II, III und IV mit Revision der Fenster, Einlage von Paraffin oder Plastikfolie Stellen einer Columella u a nur die bestehende Trommelfellperforation verschl

Ausgehend von diesen Überlegungen stellten wir uns vor allem drei Fragen, deren Beantwortung wir nach Auswertung unseres eigenen Materials erhofften

1 Tritt nach Tympanoplastik Typ I eine Innenohrstörung auf?
2 Wie häufig ist sie?
3 Was sind die möglichen Ursachen?

Die Eingriffe wurden von verschiedenen Operateuren nach der prinzipiell gleichen Technik durchgeführt. Nach Darstellung der Trommelfellperforation, evtl durch Gehörgangserweiterung und nach Kontrolle der Beweglichkeit der Gehörknöchelchenkette, wurde die Perforation mit Temporalfascie oder Fascia lata unterfüttert Überprüft wurden die Unterlagen von 930 an der Freiburger Hals Nasen-Ohrenklinik in den Jahren 1960 bis 1972 operierten Tympanoplastiken vom Typ I, von denen wir 3 Tonaudiogramme vor der Operation, 3 Wochen und 3 Monate postop auswerteten Für die Durchschnittsberechnung des Hörverlustes in dB dienten die Knochenleitungswerte bei den Frequenzen 500, 1000, 2000 und 4000 Hz, wobei aus der erhaltenen Summe das arithmetische Mittel gebildet wurde

ERGEBNISSE

Verhalten der Knochenleitungsschwellenkurve nach Tympanoplastik Typ I

a) *3 Wochen nach Operation* (Tabelle I). 930 Tympanoplastiken Typ I war bei

Tabelle I *Verhalten der Knochenleitungsschwellenkurve bei 930 Tympanoplastiken Typ I 3 Wochen postop im Vergleich zum praeoperativen Verlauf*

Zahl der Op	KL Schwellenkurve	%
757	Unverändert	81,4
158	Verschlechtert	17,0
15	Gebessert	1,6

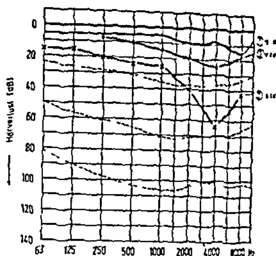


Abb 1 Knochenleitungsschwellenkurve nach Tympanoplastik Typ I Deutliches Absinken der Schwelle nach der Operation Nur teilweise Erholung bei der Nachkontrolle.

Knochenleitungsschwellenkurve gegenüber dem praeoperativen Stand unverändert 158 ≈ 17 % zeigten eine deutliche Verschlechterung dieser Schwellenkurve und bei 15 war eine Besserung gegenüber vor der Operation zu beobachten Der durchschnittliche Knochenleitungsabfall betrug zu diesem Zeitpunkt 10,34 dB, die postoperative Besserung der Innenohrleistung durchschnittlich 12,23 dB gegenuber dem praeoperativen Stand (bei 15 Fällen = 1,6%)

b) *3 Monate nach Operation* 19 Patienten waren zur Nachuntersuchung nicht mehr erschienen, so dass hier nur noch 139 ausgewertet erden konnten (Tabelle II) Davon hatte bei Patienten die Knochenleitungsschwellenkurve Ausgangswert wieder erreicht Bei 66 Patienten war eine Besserung gegenuber dem Zustand von 3 Wochen postop festzustellen, bei 8 war keine Änderung eingetreten und bei 27 liess sich eine weitere Verschlechterung aufzeigen Der durchschnittliche Knochenleitungsverlust betrug 4,86 dB

Die Nachkontrolle zeigt, dass sich in 27,4 % der Fälle der ursprunglich vorhandene postoperative Knochenleitungsverlust wieder normalisiert hat Bezogen auf die Gesamtzahl von 930 operierten Ohren bleibt 3 Monate nach der Operation eine Innenohrschädigung von 10 8 %.

Tabelle II *Verhalten der Knochenleitungsschwellenkurve 3 Monate postop bei 139 Tympanoplastiken Typ I, bei denen 3 Wochen nach dem Eingriff eine Verschlechterung der Knochenleitung festgestellt wurde*

Zahl der Op	KL Schwellenkurve	%
38	Wieder erreicht	27 4
66	Gebessert	47 6
8	Unverändert	5 8
27	Weiter verschlechtert	19 2

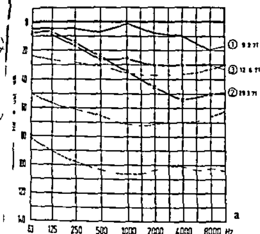
Zu ergänzen ist, dass sich unter den 930 Plastiken Typ I eine Ertaubung fand, die 2 Jahre nach der Operation auftrat und so wahrscheinlich nicht in unmittelbarem Zusammenhang mit der Operation steht

Der postoperative Knochenleitungsverlust machte sich in allen Frequenzen bemerkbar doch waren grundsätzlich die höheren Frequenzen von 2000 bis 8000 Hz stärker von der Innenohrschädigung betroffen (Abb 1)

Geschlechtsspezifische Innenohrreaktionen konnten wir ebensowenig feststellen wie vom Alter abhängige — unsere Patienten waren zwischen 7 und 70 Jahre alt — Schädigungen des Innenohres Bei Überprüfung der Unterlagen zeigte sich zwar, dass direkt nach der Operation der Knochenleitungsverlust bei älteren Patienten gegenüber den jugendlichen etwas deutlicher war, dass sich aber 3 Monate nach der Operation kein Unterschied mehr fand

Patienten mit mehrmaliger Tympanoplastik Typ I am gleichen Ohr

Bei 28 Patienten wurde die Plastik Typ I aufgrund einer Sekundärperforation einmal wieder



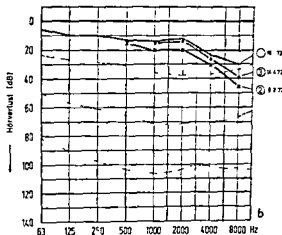


Abb 2 Pat. H P a) Tympanoplastik Typ I li Ohr (Fruhjahr 1971) Deutliches Absinken der Knochenleitungsschwellenkurve postop und partielle Erholung 3 Monate später b) Tympanoplastik Typ I re Ohr (Fruhjahr 1972) Geringes Absinken der Knochenleitungsschwellenkurve nach dem Eingriff Bei der Nachkontrolle Ausgangswert wieder erreicht

holt Nach der Operation besserte sich die Knochenleitung in 5 Fällen = 17,8 % um durchschnittlich 4,2 dB, unverändert waren 8 = 28,6 % und verschlechtert hatte sich die Hörleistung uber Knochenleitung bei 5 Patienten = 23,6 % um durchschnittlich 9,5 dB Bei 3 Patienten wurde die Tympanoplastik Typ I dreimal durchgeführt Von diesen Patienten war die Knochenleitung bis zu 1 Jahr nach der dritten Operation bei einem unverandert geblieben, bei den beiden anderen hatte sie sich um durchschnittlich 3 dB verschlechtert Die Ergebnisse zeigen, dass Mehrfachoperationen auch bei Tympanoplastik Typ I ein Risiko fur das Innenohr darstellen

Beidseits operierte Ohren

Bei 40 Patienten wurde auf beiden Ohren eine Tympanoplastik Typ I durchgeführt 5 Patienten zeigten nach der Operation auf dem einen Ohr einen Knochenleitungsverlust auf Das Gegenohr blieb nach der Trommelfellplastik ohne Innenohrschadigung (Abb 2) Bei einer anderen Gruppe von 6 Patienten war zwar nach der ersten Operation kein Knochenleitungsverlust festzustellen, beim Gegenohr trat dieser jedoch nach erfolgter Operation auf Nur in 2 Fallen konnte nach beidseitiger Operation auf jedem Ohr eine Verschlechterung der Innenohrleistung registriert werden so dass nach unseren Ergebnissen nach einem Innenohrabfall auf dem einen Ohr die nachfolgende Operation des Gegenohres nicht zwangslaufig zu einem Knochenleitungsverlust fuhrt

Vorgeschädigte Ohren

Auch die Frage, ob vorgeschädigte Ohren im Hinblick auf die postoperative Erholung der Knochenleitung ein anderes Verhalten als praeoperativ normale Innenohren zeigen, konnen wir verneinen Zu den vorgeschädigten Innenohren zählten wir jene, bei denen praeoperativ eine deutlich ausgeprägte c^5-Senke im Tonaudio-

zwischen 500 und 4000 Hz bei diesen Ohren ein um etwa 4 dB starkerer Knochenleitungsverlust als bei den nicht vorgeschädigten Innenohren vorhanden war Bei der Kontrolle 3 Monate nach Operation war dann kein Unterschied mehr zwischen vorgeschädigten und praeoperativ normalen Innenohren festzustellen Wir sind daher der Auffassung dass eine Vorschädigung ohne wesentliche Bedeutung ist da auch hier nach entsprechender Wartezeit gleiches Knochenleitungsverhalten zwischen vorgeschädigten und nicht vorgeschadigten Innenohren vorliegt

DISKUSSION

Aufgrund unserer Untersuchungen müssen wir *feststellen, dass nach Tympanoplastik Typ I* eine Innenohrschädigung unterschiedlichen Grades auftreten kann 3 Wochen postop war sie bei 17% unserer Patienten festzustellen, 3 Monate nach dem Eingriff noch bei 10,8% Wir können so die Ergebnisse von Bicknell (1971), 14%, sowie Thornburn (1971), 9%, bestätigen Befallen waren alle Frequenzen, besonders bevorzugt die höheren ab 2000 Hz Hervorzuheben ist, dass bei 1/4 der Patienten, die 3 Monate nach dem Eingriff noch eine Innenohrschädigung aufwiesen, die Hörverschlechterung 30 dB und mehr betrug

Zu beantworten bleibt die Frage nach den Ursachen dieser Beeinträchtigung der Innenohrfunktion nach Tympanoplastik Typ I Entscheidend für die Entstehung einer Innenohrschädigung bei diesem Typ der Tympanoplastik sind u E vor allem folgende Faktoren

1 Die Tatsache, dass wir oft an einem kranken oder krank gewesenen Ohr operieren, worauf auch Heermann (1960) bereits hingewiesen Ein Wiederauftreten einer entzündlichen Reaktion lasst sich nicht voraussehen und auch nicht vermeiden Sie mag moglicherweise durch den Eingriff selbst ausgelöst sein

2 Fur besonders wichtig halten wir die operative Belastung, worauf auch Kley (1969) und Schwetz (1957, 1958) sowie in jungster Zeit Palva hinweisen Manipulationen an der Gehörknöchelchenkette wie Kurzung des Hammergriffs, um iatrogene Cholesteatome zu vermeiden, oder die Prüfung der Beweglichkeit der Gehörknöchelchenkette führen zu unphysiologischen Schwingungen des Steigbugels, worauf Tonndorf (1973) erst vor kurzem hingewiesen hat Wir müssen dabei mit einer mechanischen Irritation des Innenohres rechnen, die mit dem akustischen Trauma vergleichbar ist (Palva et al, 1973)

Folgen dieser mechanischen Irritation des Innenohres sind metabolische Storungen So konnte Schnieder (1972) nach experimentellen Stapediolysen eine Eiweissentladung beobachten, und Schatzle & Haubrich (1971) stellten im Tierexperiment nach Stapedektomie Fermes schädigungen im Bereich der Sinneszellen c Basalwindung der Cochlea fest Diese Sto *wechselstörungen werden im Tierexperiment* 3 bis 4 postoperativem Tag merkbar und bil sich in vielen Fällen bis zum 28 Tag nach d Eingriff wieder zurück, ähnlich wie wir es fru nach akustischem Trauma aufzeigen konnt (Beck & Michler, 1960) Dies erklärt das Wied ansteigen der Knochenleitungsschwelle in eine späteren postoperativen Zeitraum Das were Absinken der Knochenleitung in einzelnen F len kann darauf zurückgeführt werden dass si ein Teil der geschädigten Haarzellen im Cor schen Organ nicht wieder erholt, sondern z grundegeht Es handelt sich hier um biologis Reaktionen, mit denen auch beim Typ I d Tympanoplastik zu rechnen ist Für die wi Rolle einer mechanischen Irritation und der d durch ausgelösten metabolischen Störung in d Cochlea nach Tympanoplastik Typ I spric auch unsere Beobachtung, dass nach Mehrfac operationen und damit nach mehrmaliger Tra matisierung des Innenohres ein deutlicher Kn chenleitungsabfall zu beobachten war

3 Von Wichtigkeit ist weiter die individuel Empfindlichkeit des Innenohres auf höre bessernde Operationen (Wullstein, 1968, Ba tual, 1964, Schnieder, 1972, Schwetz 19 1958) Für die Auslösung des Schadens kann i jedoch nach unseren Untersuchungen ke grundsätzliche Bedeutung zukommen Müsse doch nach Schnieder (1972), beide O gleichermassen auf den operativen Eingriff re gieren So wäre bei beidseits operierten Patien auch ein beidseitiger Innenohrschaden zu erwa ten Bei unseren beidseits operierten Patie konnten wir dagegen feststellen, dass ei Knochenleitungsabfall bei Operationen des ein Ohres bei Operation des Gegenohres nicht au treten muss Von 40 Fällen war dies nur bei Patienten der Fall

Beim Ausmass der geschilderten Folgen d mechanischen Irritation ist allerdings der ind viduellen Empfindlichkeit u E eine wesen liche Rolle zuzuweisen Jeder Operateur wei dass nach intraoperativer Traumatisierung kein

nenohrreaktion eintreten kann, während bei Eingriffen, bei denen man glaubt, besonders schonend vorgegangen zu sein, eine postoperative Innenohrschädigung zu beobachten ist.

Eine Vorschädigung des Innenohres (Boenninghaus, 1962, Schwetz, 1957, 1958) dürfte ...n nach unserer Untersuchung ebenso wie Alter und Geschlecht keine wesentliche Rolle spielen

SUMMARY

...e have evaluated the audiograms of tympanoplasties ...e I which were operated upon between 1960 and 1972 ... the ENT Clinic in Freiburg In 17 % of cases, the inner ... turned out to be impaired, 3 weeks after the operation ...ree months after the tympanoplasty 10 8 % of the inner ...s proved to be damaged We have discussed the various ...ses of these impairments 1 During the operation the ...er ear can become traumatised 2 Operations are often ...de on impaired ears. 3 There is an individual degree of ...ability in each ear

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MIDDLE EAR MECHANICS AND ALTERNOBARIC VERTIGO

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Abstract The effects of overpressure induced locally in the middle ear through free air communication between the middle ear and the external ear canal were compared with the effects of a relative overpressure of the same magnitude induced indirectly by lowering the ambient pressure The same vestibular response was seen and the main condition for inner ear stimulation was the fact that the overpressure should reach a certain level However a certain pressure difference between the ears is also involved The overpressure seemed to affect the inner ear without involvement of the eardrum, and merely a movement of the eardrum could not be shown to affect the inner ear The possibility of caloric stimulation due to temperature changes of the middle ear gas volume was also considered and excluded

[illegible] Vertigo (A V), i e vertigo due to increased pressure in the middle ear, has been studied by the present author in cooperation with Ingelstedt & Ivarsson (1974) in an experimental study on man It was found possible to elicit A V in certain subjects when exposed to simulated ascents during which the subjects were told to avoid active equilibration There was then no clearing of the ears until the relative overpressure in the middle ears was high enough to force the Eustachian tubes open passively

The way in which this relative overpressure affects the inner ear is, however, still unknown The mechanism has been assumed to be due to anatomical abnormalities such as a thin bony wall between the middle ear and the inner ear (Melvill Jones, 1957, Benson 1965) or an un-usual oval and round window anatomy" (Bo[illegible] et al , 1964) Vertigo and nystagmus caused [illegible] overpressure in the middle ear were however also demonstrated by Nylén & Karlefors (19[illegible] They induced an overpressure directly in the middle ear in subjects with an eardrum perfo[illegible]tion due to i a chronic or acute otitis med[illegible] They found that a pressure of at least 50-6[illegible] mmHg was required for the vestibular respo[illegible] to appear, as a rule, however, 100 up to [illegible] mmHg or more Yet Ingelstedt et al (197[illegible] could show that it is possible to elicit ver[illegible] in otologically healthy subjects also They could elicit vertigo in 5 out of 79 subjects but these subjects had a forcing pressure on one side of a much higher level than the mean forcing [illegible]sure of the material

Vestibular responses to ambient pressure changes have also been supposed to be due to sudden movements of the eardrum system (ear drum and the ossicular chain) causing a fluid displacement in the inner ear (Melvill J[illegible] 1957, Fields, 1958, Gregg & Ferrel 196[illegible] These suggestions, however, are not based on experiments Such sudden movements are thought to occur at the moment of pressure regulation of over- and underpressure in the middle ear or at the moment of a positive Valsalva's manoeuvre The footplate of the stapes may be expected to move in the same direc[illegible] as the eardrum and it is suggested that larg[illegible] displacements of the eardrum might cause [illegible] displacement in the inner ear through the lin[illegible]age between the stapes and the eardrum sys[illegible]

This investigation was supported by grants from the Swedish Medical Research Council (No B 73 17X 99-09) and the Swedish Delegation for Applied Defence Research (Project Nos U 87/1972 and U 95 1973)

...eriments, however, have been performed ...simulated ascents and descents of 90 cm ...) (corresponding to about 800 m ground ...level) at a speed of 4 cmH_2O/sec (about ...m sec), during which the subjects actively ...lated the middle ear pressure, thus causing ...en large displacements of the eardrum No ...er ear stimulation could be recorded during ...se experiments (Ingelstedt et al , 1974) Yet ...other experiments on the same subjects these ...hors were able to show that it was possible ...elicit A V without sudden pressure changes ...ey found a vestibular stimulation starting ...ing a relative slow pressure increase in the ...ddle ear (4 cmH_2O/sec), but the stimulation ...ld not be recorded until the relative over- ...ssure reached a certain level (about 60 cm ...O) Asymmetry between the ears owing to ...ferent forcing pressures could also be ob- ...ved

The aim of the present study was to in- ...stigate

(*a*) if the vestibular stimulation is elicited by ...vements of the eardrum when the relative ...erpressure in the middle ear displaces the ...drum outwards, thereby moving the stapes ...erally

(*b*) if the relative overpressure in the middle ...r acts upon the inner ear directly without in- ...olvement of eardrum movements

MATERIAL

The material consisted of 2 subjects, 23 and 25 years old. The subjects were regarded as otologically normal with no history of ear disease and a normal ear examination The hearing threshold was within 0–20 dB (related to ISO standard 1964) The subjects were free from signs of catarrhal infection and there was no story of labyrinthine disease The vestibular function was tested by caloric irrigation and ENG, and was found normal Only 2 individuals were examined since it was difficult to find suitable subjects The criteria for the selection of the subjects were as follows

Otologically healthy, high forcing pressure on one side, appearance of vertigo when exposed to simulated ascents and passive clearing of the ears

Subjects with the above mentioned qualities had to agree to myringotomy and application of a transmyringeal plastic tube

The subjects were selected from the material of another study, presented by Ingelstedt et al (1974), (subjects I and II)

EQUIPMENT AND METHOD

The following symbols are used

P_{atm} atmospheric pressure on ground
P_m pressure in middle ear
P_{tm} pressure gradient across eardrum
P_{ch} pressure in chamber, i e ambient pressure
P_{ec} pressure in external ear canal
V_m volume of airfilled middle ear space
V_{tm} volume displacement of eardrum in relation to its neutral position
V_{muc} volume of mucous membrane lining the middle ear space
$\dot{V}_{tm}$ airflow through resistor of the ear canal flow meter, caused by volume displacement of eardrum
$\dot{V}_{ec}$ airflow through resistor of the ear canal flow meter, caused by expansion and compression of gas volume in external ear canal and in flowmeter system by changing the ambient pressure
$\dot{V}_{ref}$ airflow through resistor of the reference flowmeter, caused by expansion and compression of gas volume in reference system by changing the ambient pressure

Δ before the symbol indicates a change of the variable

Pressure is expressed in cmH_2O, volume in microlitre (μl) or millilitre (ml), and airflow in microlitre sec (μl sec)

P_m P_{ch} and Pec are relative to atmospheric pressure on the ground
P_{md} middle ear pressure minus the pressure of saturated water vapour at 37 C, this being regarded as non-compressible according to Boyle s law

The effect of overpressure in the middle ear was studied by increasing the pressure in the middle ear in different ways, indirectly and directly

I *Pressure gradient across an intact eardrum by changing ambient pressure*

Fig 1 gives an outline of the equipment used for the recordings The middle ear pressure

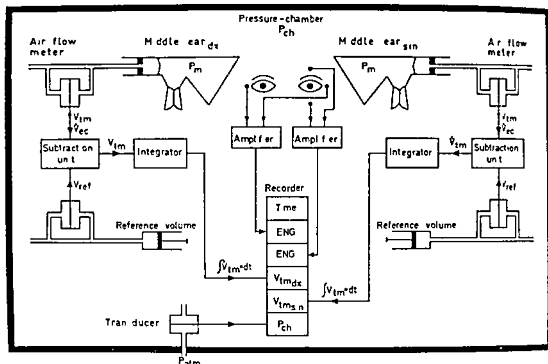


Fig 1 Outline of the equipment used for recordings of the displacement of the eardrums (V_{tm}) at changing ambient pressure (P_{ch}) The equipment as well as the subjects are placed in a pressure chamber For further details see text and list of symbols

increased indirectly by lowering the ambient pressure (simulated ascent) and the subjects were instructed to avoid active equilibration A pressure chamber was used in which it is possible to simulate ascents of 90 cmH_2O in 25 sec, with a constant rate of pressure decrease (4 cmH_2O/sec) during 90% of the time The induced relative overpressure in the middle ear caused an outward displacement of the eardrum A method was used (Ingelstedt et al, 1967, Elner et al, 1971) by which it is possible to record the volume displacement of the eardrum (V_{tm}) in relation to its neutral position at changing ambient (P_{ch}) or middle ear (P_m) pressure

A polyethylene catheter with its end passing through a rubber disc was inserted into the inner bony part of the external ear canal airtightly connecting the space between the eardrum and the rubber disc with the measuring device There was a free air communication between the outside of the eardrum and the ambient pressure, and the airflow caused by the eardrum displacement ($\dot{V}_{tm}$) was recorded by means of a flowmeter The flow $\dot{V}_{ec}$ has to be eliminated for an isolated recording of $\dot{V}_{tm}$ during ambient pressure changes This elimination is possible by the use of an identical flowmeter system adjusting a reference volume identical with the ear canal volume Then the flow signal ($\dot{V}_{tm}$) is integrated and Vtm is recorded Fig 2 gives an example of a recording

From these recordings of V_{tm} and P_{ch} it is possible to calculate the pressure gradient across the eardrum (P_{tm}), i e the relative overpressure in the middle ear, at the moment when the onset of the vestibular response (nystagmus) was recorded These calculations were made as described by Ingelstedt et al (1974) For further details of equipment and method see Ingelstedt et al (1967) and Elner et al (1971)

II *Pressure gradient across an intact eardrum by change of pressure in the external ear canal*

The pressure in the external ear canal (P_{ec}) on the one side was lowered with a pressure

Recording of eardrum volume displacement (V_{tm} and chamber pressure (P_{ch} in cmH_2O) Eardrum nent outwards (+) and inwards (−) Each exam n starts with a simulated descent with a pressure of +45 cmH_2O It is then checked that the ear- is in its neutral position (V_{tm}=0) after active oration The simulated ascent then starts (*a*) with ssure decrease of 90 cmH_2O The Eustachian tube ned passively (passive forcing), and the eardrum inwards to the neutral position when the middle essure is equilibrated to the chamber pressure by itions (*arrows*) When the chamber pressure is in- (*b*) the eardrum moves inwards and when the e ear pressure is equilibrated by deglutitions (*ar-* , the eardrum again moves to its neutral position

htly connected to the external ear canal as n in Fig 3 By this method it was possible use an outward displacement of the ear- 1 as mentioned above

Overpressure in the middle ear with ressure gradient across the eardrum an immobile eardrum

1 the equipment shown in Fig 3, the middle pressure (P_m) was increased directly via free ommunication between the middle ear and external ear canal through a transmyringeal (inner diameter 0 8 mm) (Fig 3*b*) During nvestigations eye-movements were recorded ltaneous with the pressure recordings by ns of electronystagmography (ENG), and recordings were performed in total darkness the subject's eyes open (Tjernström, 1973) flow-volume measuring unit and the pres- measuring unit (cf Figs 1 and 3) were rated before and after every examination rding to the original method Eye-move- ts were calibrated for 30° in each direction re and after every test

EXPERIMENTAL PROCEDURE

A *Before myringotomy*

(1) The subjects were exposed to simulated ascents in the pressure chamber and the volume displacement of the eardrum (V_{tm}) was recorded during passive forcing of the Eustachian tubes from both sides, together with ENG-recording of eye-movements The subjects were examined while seated and were instructed to keep their eyes open in total darkness and to report onset of vertigo

(2) Underpressure was induced locally in the external ear canal with the equipment shown in Fig 3 The pressure was controlled so as to cause an eardrum movement outwards of the same degree as that caused in the experiments described above (procedure A 1)

B *After myringotomy and application of transmyringeal tube*

Myringotomy was performed on the side with the highest forcing pressure, which was found to be on the right-hand side in both subjects

(1) The subjects were exposed to simulated ascents and passive forcing of the left Eustachian tube, while the pressure in the right middle

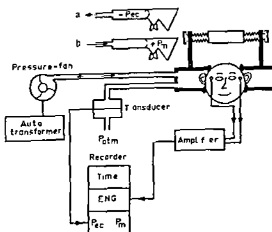


Fig 3 Outline of the equipment used to induce over- and underpressure in the external ear canal (*a*) Negative pressure in the external ear canal (P_{ec}), (*b*) over pressure in the middle ear (P_m) through a perforation in the eardrum The pressure fan is connected to the external ear canal by a plastic cylinder airtightly fixed around the subject's external ear

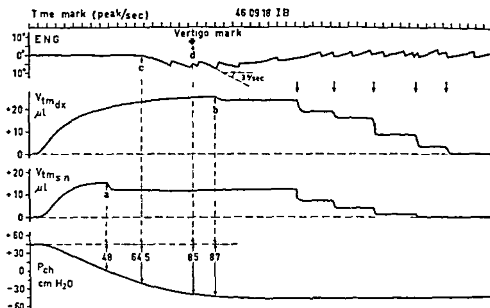


Fig 4 Electronystagmogram (ENG), volume displacements (in µl) of right ($V_{tm_{dx}}$) and left ($V_{tm_{sin}}$) eardrums and chamber pressure (P_{ch} in cmH$_2$O) during passive forcing of the Eustachian tubes Arrows mark deglutitions Passive clearing of the left ear (*a*) and right ear (*b*) Nystagmus starts when the ambient pressure changed by 64 5 cmH$_2$O (*c*)—slow nystagmus The subject reports vertigo (*d*) when the chamber pressure is lowered by 85 cmH$_2$O For more comments see text

ear followed the chamber pressure ($P_m - P_{ch}$) owing to the transmyringeal tube

(2) With the equipment shown in Fig 3 overpressure was induced locally in the right middle ear via the transmyringeal tube The pressure level and the speed of the pressure change were made identical with those recorded in the experiments described above (procedure A 1)

RESULTS

Figs 4, 5 and 6 are recordings taken from the different experimental situations on subject I The examinations of the subject II showed compatible results

A *Before myringotomy*

(1) Simulated ascents with passive clearing of the ears elicited vertigo and nystagmus in both subjects Fig 4 shows that the nystagmus started when the ambient pressure was lowered by 64 5 cmH$_2$O The calculated relative overpressure in the right middle ear, i e the pressure gradient across the eardrum ($P_{tm_{dx}}$) was at that moment 59 8 cmH$_2$O (NPL = nystagmus pressure level) and the pressure difference between the right and the left ear at the same moment was 42 5 cmH$_2$O The NPL for subject I was between 59 8 and 64 1 cmH$_2$O, and the pressure asymmetry between 42 5 and 47 2 cmH$_2$O (3 examinations) Corresponding data for subject II was NPL = 54 and asymmetry [illegible] cmH$_2$O

(2) Underpressure locally in the right external ear canal (P_{ec}) caused an eardrum movement outwards The speed and the magnitude of the pressure decrease was controlled so as to give an outward movement of the same degree as in the experiments with passive forcing of the tubes The subjects reported no vertigo and no nystagmus was recorded

B *After myringotomy and application of transmyringeal tube*

(1) The subjects were exposed to simulated ascents without active clearing of the ears. During the ascents a relative overpressure was built up only in the intact left ear causing a pressure asymmetry between the ears As is seen in Fig 5 about the same asymmetry was

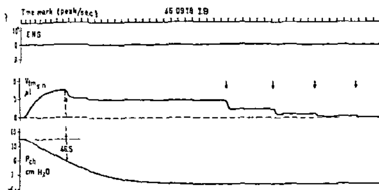


Fig 5 Electronystagmogram, volume displacement of left eardrum ($V_{tm_{sin}}$ in μl) and chamber pressure (P_{ch} in cmH_2O) during passive forcing of the left Eustachian tube A transmyringeal plastic tube in the right ear prevented pressure gradients across the ear drum on that side

as that recorded in the experiments with passive forcing of both Eustachian tubes (cf. Fig 4) The calculated forcing pressure (FPL = forcing pressure level), $P_{tm_{sin}}$ was 42 6 cmH_2O Despite the same asymmetry no vertigo appeared and no nystagmus was recorded Thus asymmetry *per se* is not sufficient to cause vertigo

(2) Overpressure was induced locally in the external ear canal of the ear with the transmyringeal tube (right ear) Owing to this tube the pressure in the middle ear (P_m) followed the external ear canal pressure without any displacement of the eardrum The speed of the pressure change was controlled so as to be the same as that recorded at the passive forcing of the tubes with intact eardrums, and the pressure increase in the external ear canal was interrupted before the tube was forced open passively in order to avoid airflow through the middle ear, which could otherwise cause caloric stimulation As is seen from Fig 6 it was possible to record nystagmus of about the same intensity and starting at about the same pressure level as was seen in the experiments with passive clearing of both ears (cf Fig 4) The subjects also reported vertigo of about the same intensity as that at the experiments with passive clearing of the ears

DISCUSSION

The results of the present study provide further evidence that a relative overpressure in the middle ear may stimulate the vestibular system The overpressure was induced in two different ways, by lowering the ambient pressure (intact eardrum) or by overpressure induced locally via a transmyringeal tube (direct to the middle ear) Roughly the same vestibular response was seen despite the different ways of inducing the relative overpressure, and a prerequisite for inner ear stimulation seems to be that the overpressure should reach a certain level The pressure level is also found to be of a magnitude that

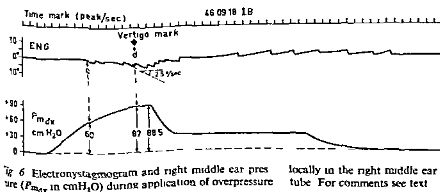


Fig 6 Electronystagmogram and right middle ear pressure ($P_{m_{dx}}$ in cmH_2O) during application of overpressure locally in the right middle ear through a transmyringeal tube For comments see text

might occur during flight and diving It is seen from the present work that movements of the eardrum in connection with the ossicles *per se* do not affect the vestibular system It is also seen that approximately the same vestibular stimulation was recorded under the two different experimental conditions with overpressure in the middle ear although the eardrum was immobilized by a transmyringeal tube in one of the situations

Pressure changes might affect the inner ear indirectly by temperature variations (caloric stimulation) caused by expansion or compression of the middle ear gas volume due to pressure variations in the middle ear (ΔP_m) To exclude the possibility of a caloric stimulation it is necessary to discuss the pressure changes in the middle ear in the various experimental situations

In the experiments with simulated ascents and passive clearing of the ears (cf Fig 4) there is a great relative overpressure in the right middle ear (P_{tmdx}) before the Eustachian tube is forced open, and the inner ear stimulation is found to start when the relative overpressure has reached 59 8 cmH_2O The absolute pressure change in middle ear (ΔP_m) at this moment has then reached 4 9 cmH_2O This pressure change can be calculated by using Boyle s law according to Ingelstedt et al (1967 and 1974), and Andreasson (1973)

$$\Delta P_m = \frac{\Delta V_{tm} + \Delta V_{muc}}{V_m} P_{md}$$

The following factors are required for this calculation

ΔV_{tm} the recorded eardrum displacement outwards on the right hand side (in the example given in Fig. 4 subject I – 22.4 μl)

ΔV_{muc} – the calculated volume change of the middle ear mucosa (0 5 μl cmH_2O) Andréasson et al, 1974) (in the example given in Fig 4 subject I – 29 9 μl)

V_m – the middle ear volume calculated by using a roentgenological plan metrical method which means transferring the area of the air filled middle ear system, measured from X ray films to a volume according to Andreasson (1973) (in subject I – 10 900 μl)

In some experiments, overpressure was induc- locally in the middle ear through a transmyrin- geal tube (cf Fig 6) In a comparison of the ex- perimental situations with an intact and a per- forated eardrum approximately the same rela- tive overpressure (59 8 resp 60 cmH_2O) was re- corded at the moment nystagmus started (Figs 4 and 6), but the absolute pressure chan- (ΔP_m) was different, 4 9 resp 60 cmH_2O Ap- proximately the same inner ear stimulation wa- however, recorded Exclusion of caloric stim- ulation in the above mentioned situations further confirmed by the experiments in a transmyringeal tube was applied on one sid Then ΔP_m *during ascents follows* ΔP_{ca} with a range of 90 cmH_2O Despite this great re- lateral pressure change no inner ear stim- tion was recorded Thus the vestibular stim- tion does not seem to be caused by a ture change

Is it possible that the overpressure might fect the vestibular system directly via the and/or the round window by means of an ear fluid motion (endo- and perilymph) ca- ing a cupula deviation? It has been shown several authors many years ago, in tions of preparations from homo (e g Bezold 1908, Nylen, 1923) and of living animals (K rak, 1935), that the labyrinthine pressure rise or fall according to pressure changes the middle ear Inner ear fluids might be set in motion by overpressure in the
in different ways, for instance by a fluid di-
placement between the two windows of
type caused by acoustic vibrations However
it is difficult to understand such
an overpressure in the m
equally on all resiliant structu
two windows The pressu
however, owing
brane of the round
plate From the
seen that the
pressure chiefly
the round window,
greater than that
also possible

stapes could even move the stapes against pressure gradient when the eardrum is dis-ed outwards, but the function of the ossic-r chain in such circumstances is not exactly wn

nother factor responsible for inner ear fluid on might be a fluid displacement via the lymphatic and perilymphatic ducts, but ions differ as to the patency of these ducts lefors, 1924, Waltner, 1948, Lempert et al, Allen, 1964, House, 1964, Ritter & Law- 1965 Anson, 1965, Holden & Schu-ht, 1968)

nally, the vestibular system might be af-d by a circulatory disturbance causing aemia due to venous or capillary stasis ed by the increased middle and inner ear ure A venous stasis together with a lym-c stasis might also cause a further intra-nthine pressure increase interfering with irculation of the inner ear fluids Blood l connections between the middle and the ear have been shown to exist (e g Hansen, and the pressure effect on the middle ear sa might be transmitted to the inner ear ese connections

view of these various mechanisms which been discussed, the above-mentioned work ylen & Karlefors (1921) is of particular est These authors could show that a con-overpressure in the middle ear, in sub-with an eardrum perforation, caused gmus which gradually increased in strength g 10–20 seconds and then ceased In their on the mechanism is an "impression on elding portions of the labyrinth, which d cause a lymphatic movement with ac anying irritation

ZUSAMMENFASSUNG

bsoluter Überdruck wurde mit freier Luftzufuhr den Gehorgang im Mittelohr hervorgerufen und in Wirkung mit relativem Überdruck gleicher nordnung verglichen Der relative Überdruck indirekt durch Senkung des Umgebungsdruckes gerufen Dieselbe vestibulare Reaktion konnte n genannten unterschiedlichen Methoden hervor n werden unter Voraussetzung dass der Überdruck ein gewisses Mindestmass erreichte Ein gewisser Druckunterschied zwischen beiden Mittelohren ist an der Wirkung auf das Vestibularorgan mitbeteiligt. Der Überdruck ubt seine Wirkung ohne Anteilnahme des Trommelfells aus, blosse Bewegungen des Trommelfells hatten keinen Einfluss auf das Innenohr Die Moglichkeit eines kalorischen Reizes hervorgerufen durch Temperaturschwankungen im Gasvolumen des Mittelohres wurde beachtet und konnte ausgeschlossen werden

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INVESTIGATION OF POSITIONAL AND POSITIONING NYSTAGMUS BY USING A NEW TYPE OF ELECTRICALLY-DRIVEN ROTATION TABLE

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...ract The different methods for investigating positio- ...nd positioning nystagmus are presented and dis- ...ed The latest results concerning the interaction be- ...n the utricles and the horizontal semicircular canals ...iven and based on these findings, a new electrically- ...n rotation table for investigating positional and ...oning nystagmus is demonstrated. The mode of ...lation and the predictable, normal results are pre- ...d.

...stigation of positional nystagmus can be ...ied out in several different ways, with the ...ent in either a sitting or a supine position ...ally the investigation is performed in two ...rent planes

...he sagittal plane, sitting, supine or with the ...ead hanging out over the upper edge of the ...ble

The frontal plane, right or left lateral posi- ...on with supine position as the starting posi- ...on

...special kind of positional nystagmus is the ...alled positioning nystagmus, i e nystagmus ...ediately after taking a certain position ...ger (1955) recommends a rapid alteration ...osition in the sagittal plane, from sitting to ...e, with head hanging and back again to a ...g position By this means a transitory, ...lly rotatory or horizontal-rotatory nystag- ...is obtained, which lasts between a few sec- and 1½ min The nystagmus is usually ...eproducible Another procedure is used by & Hallpike (1952) who within 3 sec changed ...atient s position i sitting to supine with the head simultaneously turned 45° to either side This, as a rule, induces a rotatory nystagmus of short duration

However, the way in which these tests are performed always involves a risk of interference from other sensory organs Turning the head in relation to the shoulders also stimulates the so-called neck reflexes Therefore Nylen (1950) recommended that head and body should be moved together However, the importance of this was denied by Cawthorne (1954)

The movement factor is also of great importance In order to be able to term the findings positional nystagmus, it is necessary to exclude every kind of interference from the semicircular canals, which may be stimulated if the angular moment is too large

Attempts have been made to eliminate these false sources by constructing special posture-tables Such mechanically-driven tables have been constructed by Quix (1926), Grahe (1927) and Fromm & Nylen (1935) Though this has certainly eliminated the problem of neck reflexes the manual drift of the table has not been controllable or reproducible Consequently, Aschan et al (1956) constructed an electrically-driven posture table, which could accelerate by 0 05–0 1°/sec² up to a speed of 1–3°/sec Although this type of table has made it possible to make interesting studies on positional nystagmus, owing to its limited possibilities, it cannot be used for investigations ...

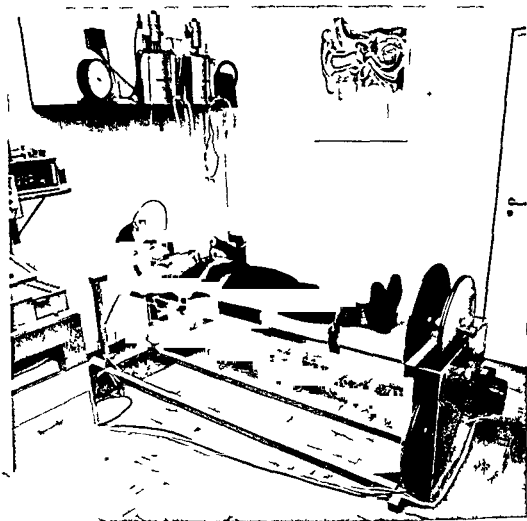
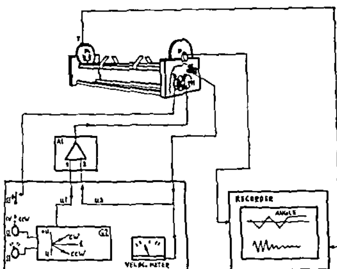


Fig 1 The rotation table

used for systematic studies on the interaction of utricular and semicircular-canal activities As the function of the otolith organs was not known in detail in the mid 1950s especially regarding the oculomotion the findings reported were of limited value However investigations made during recent years have thrown new light on this problem Fluur & Mellstrom (1970) have shown that electrical stimulation of different areas on the utricular surface induces distinct eye movements in varying directions depending on the area stimulated Subsequently Fluur & Siegborn (1973*a b c*) in the cat and Fluur (1973) in man have shown that these different areas cooperate with the semicircular canals in an intimate and systematic way in their influence on the oculomotor system The results sl o v that

the horizontal nystagmus obtained after u lateral labyrinthectomy is facilitated if th tient or the animal is tilted around h s (longitudinal axis towards the diseased ear is inhibited if the tilting is towards the sound From these experiments it is concluded utricle and the ipsilateral horizontal sem c lar canal function synergistically whereas utricle and the contralateral horizontal sem cular canal function antagonistically Theref a right beating nystagmus becomes more in a left lateral position than in a right

The above-mentioned results can now be u clinically to investigate positional and pos ing nystagmus as an expression of the i action between the otolith organs and the se circular canals and especially between the u



A1	Amplifier	P	Angle potentiometer
B	Brake	S1	Acc/Dec value switch
G1	Tachogenerator	S2	CW/CCW acceleration switch
G2	Rampgenerator	S3	Brake switch
M1	Table drive motor	T	Connection for electrodes

Fig 2 Schematic picture of the electronic equipment

and the horizontal semicircular canals The reason why the horizontal semicircular canals have been chosen is partly because a modulation of horizontal nystagmus is as good a parameter of the utricular function as the vertical or rotatory nystagmus, and partly because horizontal nystagmus is the easiest to record electronystagmographically

THE ROTATION TABLE MSA[1]

In order to make these investigations possible we have constructed in collaboration with MSA (Stille–Werner AB, Stockholm, Sweden) an electrically-driven rotation table with wide variability in the stimulation pattern

To a steel stand is attached a bunk 2 × 0 5 m, which can be rotated around a horizontal longitudinal axis The subject's head can be placed in a head holder provided with two occipital and two temporofrontal pads, the latter are attached to the head by means of a screw so that it is firmly fixed The body is fixed to the bunk partly by two lateral thoracic supports, and partly by four safety belts around the body and the bunk (Fig 1)

In order to ensure that the table functions without causing vibrations and other disturbances it is driven by an electrically controlled servosystem, which operates as follows (Fig 2) The angular velocity of the driving motor M1 is determined by d c voltage U1, which represents the value of the desired velocity U1 is fed into input 1 of the amplifier A1 The angular velocity of the driving motor is measured by the tachogenerator G1, which produces a d c output voltage U2 proportional to the angular velocity of the driving motor Thus, U2 represents the value of the actual velocity U2 is fed into input 2 of the amplifier A1, where it is compared with the control voltage U1 If there is a difference between the actual velocity and the desired velocity, there will of course be a corresponding difference between U2 and U1 The amplifier will then produce an output signal which will change the angular velocity of the motor until U2 equals U1

If the control voltage U1 is increased linearl with time, a constant acceleration of the

[1] MSA = Medicinska Servosystem AB

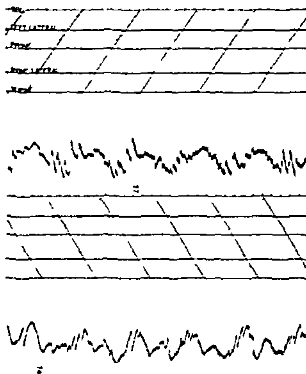


Fig 3 Electronystagmographic recordings from a normal person during constant rotation at a speed of 60 sec around the longitudinal axis The upper half of the curves indicates the rotation of the table the lower half indicates the horizontal nystagmus The upper curves show nystagmus towards the right during clockwise rotation inhibited in the right lateral position and facilitated in the left position The lower curves show nystagmus to the left during counterclockwise rotation inhibited in the lateral position and facilitated in the right lateral position

of the table is obtained If U1 is decreased linearly with time constant deceleration is obtained

U1 is generated in the ramp generator G2, which can be set to deliver the desired change in voltage per unit time At any time desired switch 2 can be used to set the ramp generator so that the voltage-change process is stopped As U1 then becomes constant the motion of the table will stop accelerating and constant angular velocity will be maintained

The table can be brought to a standstill by controlled deceleration or by activation of the brake B by the switch S3 When the brake is actuated the voltage from the ramp generator automatically falls to zero and consequently the driving motor is stopped

Performance

1 The table can be accelerated or decelerated from 0 to 20 /sec² both clockwise and counterclockwise

2 It can be rotated at constant speed [illegible] to 100°/sec

3 It can be stopped instantly in every desired position

4 It can be coupled free and turned around manually if a certain initial or basic position is desired

Recording

The movements of the table are recorded by potentiometer P, situated on the rotation axis which indicates both motion of the table [illegible] angular position

The eye movements are recorded el[illegible] mographically with electrodes for hori[illegible] nystagmus, caudal to both lateral can[illegible] order to prevent artefacts due to [illegible] from the temporo-frontal head pads. [illegible] appropriate with a warning In the supine position the utricles are depressing the eyes, [illegible] a negative effect on the horizontal nystagmus Therefore the patient is told to look straight forward during the recording The [illegible] electrode is placed in the middle of the fore[illegible] The signals from the electrodes are conveyed to the terminal strip T and pass via slip rings to the recorder

Mode of stimulation

To investigate positional nystagmus the patient is rotated at constant speed 0 1-0 5 sec [illegible] the supine position to the right or left lateral position in which the table is stopped for 1 [illegible]

To investigate positioning [illegible] s the p[illegible] tient is accelerated clockw[illegible] celeration up to a speed [illegible] the table is driven at [illegible] During this period in [illegible] a postacceleratory [illegible] served which lasts [illegible] changes intensity [illegible] the head in [illegible] reason for this [illegible]

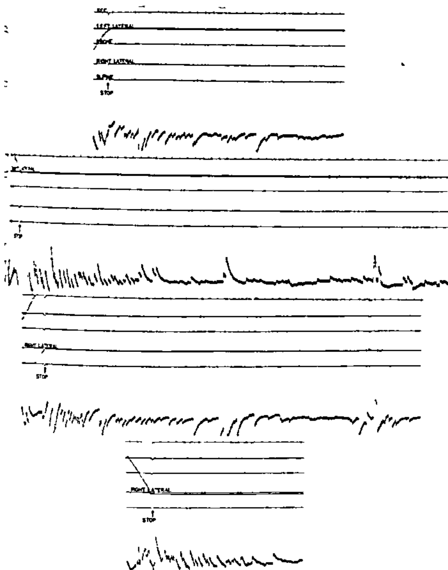


Fig 4 Electronystagmographic recording of postrotatory horizontal nystagmus from a normal person when rotation at a speed of 60 /sec is suddenly stopped in left (curves 1 and 2) or right (curves 3 and 4) lateral position after clockwise (curves 1 and 3) or counterclockwise (curves 2 and 4) rotation The upper half of one curve shows the position of the table, the lower half the direction and intensity of nystagmus In the positions where the utricles and the semicircular canals are functioning synergistically the duration of postrotatory nystagmus is much longer—about twice—than in the positions where they are functioning antagonistically

here the right beating nystagmus, induced by clockwise acceleration, is facilitated by the right utricular input, i e in the left lateral position, there is a distinct increase in intensity of the nystagmus, whereas in the right lateral position, where the increased activity from the left utricle is functioning antagonistically to the right horizontal semicircular canal, an inhibition of nystagmus is often observed (Fig 3) The table is then suddenly stopped in the left lateral position and the duration and intensity of the postrotatory left-beating nystagmus is studied Because the increased activity of the right utricle in this position functions antagonistically to the in-

creased activity of the left horizontal semicircular canal, the duration of the nystagmus will be short (Fig 4)

A few minutes later the patient is accelerated *counterclockwise in the same way as earlier* The left-beating nystagmus, which the patient most often has even during constant speed, is then studied Here an increased intensity of nystagmus is observed in right lateral position, which is due to synergism between the increased inputs from the left utricle and the left horizontal semicircular canal In the left lateral position nystagmus is inhibited due to antagonism between the increased activities of the right utricle and the left horizontal semicircular canal The table is then stopped, also this time in the left lateral position, and the duration of the postrotatory right beating nystagmus is recorded Here the right utricle functions synergistically with the increased input from the right horizontal semicircular canal Therefore, the duration of the nystagmus becomes considerably longer than that induced by the former stimulation (Fig 4), despite the patient's position being the same in both situations

The investigation is then continued again with ...ockwise acceleration constant speed and sudden stop, but this time in the right lateral position The left beating postrotatory nystagmus is here prolonged by facilitation due to synergism in input from the left utricle and the left horizontal semicircular canal (Fig 4) Finally, the patient is accelerated counterclockwise, the variations in nystagmus during constant speed are studied and the table is stopped again in the right lateral position The right-beating postrotatory nystagmus due to increased input from the right horizontal semicircular canal is inhibited here by the antagonistically operative increased activity of the left utricle, and the duration of the nystagmus is shorter than formerly in the same position (Fig 4)

ZUSAMMENFASSUNG

Die verschiedenen Methoden fur die Untersuchung von Lage und Lagerungsnystagmus sind dargestellt und diskutiert worden Die letzten Resultate der Zusammenarbeit zwischen Utriculus und den horizontalen Bogengangen werden nachgewiesen, und auf grund der Resultate wird ein neuer, elektrisch getriebener ... tionstisch fur die Untersuchung von Lage- und Lagerungsnystagmus demonstriert Die Art der S... und die erwarteten normalen Ergebn... legt

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OPTOKINETIC-GRAVICEPTIVE INTERACTION IN DIFFERENT HEAD POSITIONS

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Abstract With increasing lateral tilt of the head (0°, 30°, 45°, 60°) the apparent vertical (AV) is progressively inclined in the same direction Simultaneously, the variance of settings of a test edge increases, consistent with the theory of a progressive decline in the effectiveness of the statolith organs The AV can also be influenced by moving visual scenes rotating around the observer's line of sight It has been argued that visual motion information, which also results in apparent body tilt, contributes to the central computation of orientation of the gravitational vector If with lateral head tilt graviceptive sensory input is less accurate and less powerful the visual influence should become more potent. Indeed, displacements of AV induced by exclusive visual stimulation with the head erect are moderate but increase progressively with larger head tilt angles The potentiation of the net visual effect on AV in an inclined head position is maximal if the visual stimulus is moving opposite to the head tilt The demonstrated visual graviceptive interaction seems to have functional implications for postural orientation

Gravitational orientation depends on two major sources of experience, the exteroceptive visual and the proprioceptive postural The latter is mainly provided by graviceptive information from the vestibular maculae but also from somaesthetic gravireceptors in the trunk Although orientation depends on these distinct sources of information, we do not have in our experience distinct conceptions of the upright, one visual and the other postural Our experience of spatial orientation is unitary The final processes to which both visual and postural factors contribute produce the experience of a single upright (Asch & Witkin, 1948*a*)

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The effects of the observer's visible surroundings on his spatial orientation have been extensively studied Most investigations have been concerned with the effects of steadily tilted contours or panoramas on the apparent visual and postural vertical Although controversial in some respects, the results generally agree that the apparent vertical is moderately displaced in the direction of tilt of the visual scene (literature reviewed by Howard & Templeton, 1966) It has been reported that a visual surround continuously rotating around the observer's line of sight induced much stronger tilts of the apparent upright ranging up to 40° (Dichgans et al, 1972) and affects body posture (Brandt et al, 1973) Since the moving visual display did not entail cues to visual orientation that would conflict with those of gravity it was concluded that motion as such caused the perceived tilt The fact that the tilt was common to both visual and postural orientation led to the hypothesis that the shift occurs in the internal representation of the gravity vector It was assumed that the subjective orientation of the gravity vector is centrally computed from inputs originating in gravireceptors and the retina (Dichgans et al, 1972) The limitation of visually induced tilt was thought to be due to conflicting information given by excitation of the otolith and gravireceptive pressure receptors

Indications of a different effectiveness of statolith organs in different head position

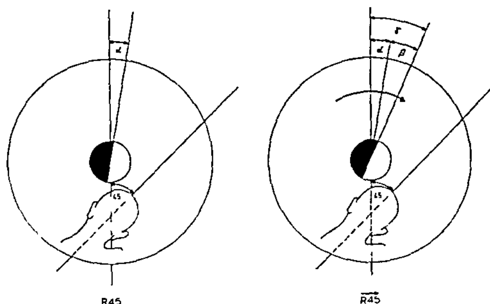


Fig 1 Schematic drawing of the different angles measured On the left head inclination only On the right with additional rotation of the visual display For further explanation see text

Colenbrander, 1963/64, Graybiel & Clark, 1962, Graybiel & Pattersson, 1955, Hofman, 1910, Nagel, 1898, Quix & Eijsvogel, 1929, Schöne, 1964) found signs of increase in variance of graviceptive information with increase of body tilt Others, more specifically, demonstrated a decrease in significance of statolith inputs concomitant with an increase in significance of visual cues for orientation of the apparent vertical when the head was progressively inclined and steadily tilted lines were presented at the same time (Asch & Witkin, 1948*b*, Bischof & Scheerer, 1970, Schöne & Udo de Haes, 1971). Young (1971) assumed a "confidence factor" for weighing statolith information at different head positions and consequently a changing nature of visual-vestibular interaction in perception of orientation

Young's hypothesis was tested and confirmed in experiments where the subject viewed a large visual pattern rotating around his line of sight while his head was held in different positions The subjects of Young et al (1974) reported that the visually induced tilt effect increased markedly for the head tilted laterally 90° or inverted These observations were confirmed in the series of experiments presented in this paper by measuring the magnitude of induced tilt obtained when the head was upright or tilted laterally 30, 45 or 60°, respectively The results not only confirm the assumption of a different weighting coefficient for graviceptive information depending on the head position in space but also its dependence on the directions of head inclination and movement of the visual display

METHODS

The experimental apparatus

A large display that could be rotated about the observer's line of sight was mounted in front of the subject Its surface was covered with a coloured floral pattern that by itself entailed no cues to orientation The disk, subtending 1[illegible] of visual angle, was viewed binocularly The rest of the visual field was masked off with [illegible] of dark cardboard The shaft mounted at the centre of the disk was connected to a d c [illegible] As in earlier experiments (Dichgans et al, 19[illegible] Held et al, in press) a smaller disk—referred to as the target disk—was mounted on a coaxial shaft just in front of the large disk The [illegible] target disk subtended 18° of visual angle and carried a straight edge that divided its surface

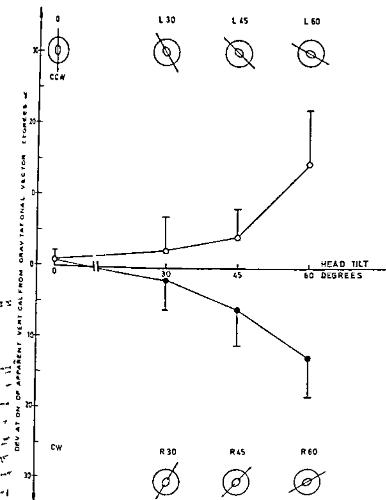


Fig 2 Deviation of apparent vertical (AV) from gravitational vector at different head positions (α) to the left (upper half) and to the right (lower half of the graph) Means and standard deviations

into black and white semicircular areas and a fixation point at the midpoint of the edge The orientation of this edge could be controlled by the observer by turning a potentiometer that in turn controlled the position of a servo-motor connected to the shaft of the target disk The shaft was also connected to a second potentiometer which linearly varied a voltage recorded on a strip chart The resolution of the measurements thus taken was 1°

Each subject was seated with the midpoint between his eyes aligned with the axis of the visual display His head was rigidly attached to a head holder by means of a bite board and by belts fastened around his occiput The orientation of the head could be altered by tilting the head holder about the sagittal axis In every new position the accuracy of alignment of the subject's line of sight with the display axis was controlled and corrected if necessary

The experimental procedure

The orientation of apparent vertical (AV) with respect to gravity was repeatedly measured in 3 experienced subjects with the head erect and the head inclined 30, 45 or 60° towards the right (R) or left (L) shoulder At every head position measurements were alternatively taken with the visual stimulus stationary ($N = 20$, in each subject) or moving at 60°/sec clockwise (→) or counterclockwise (←) ($N = 10$)

The sequence of head tilt angles and directions of disk rotation was in random order The r

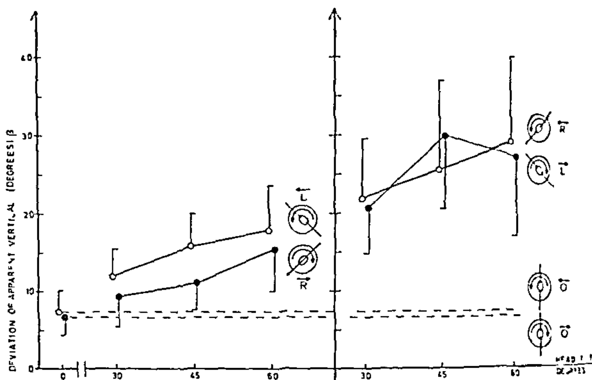


Fig 3 Additional deviation of apparent vertical induced by the rotating visual display (β) at different angles of head inclination (abscissa) Disk rotation and head inclination towards the same direction (left graph) and in opposite directions (right graph) ●, AV to the right O, AV to the left The dashed lines depict the deviation with the head erect and only the disk moving to the left and right respectively Means and standard deviations

ing tilt angles of AV with respect to gravity were called α (Aubert or Muller phenomenon) if the visual stimulus was stationary and γ if the disk was moving The net tilt effects induced by the moving stimulus were then calculated as β by subtracting α from γ (Fig 1) Means and standard deviations were determined for each of the three angles in each of the 7 head positions Means of angles were calculated after putting a negative sign to every tilt of AV that was opposite to the head tilt (Muller phenomenon) These experiments followed a pilot study that yielded consistent results In this study 40 inexperienced students had been tested with the head erect and tilted 45 to either shoulder But in contrast to the main experiment, reported here, measurements with the head tilted were taken only once and with one head tilt direction from each subject thus providing independent samples (Diener, thesis, in preparation)

RESULTS

Deviations of apparent ver l from or of gravity in different

The tilt angles of AV w of the head towards tl Fig 2 The data they are totally earlier observat Templeton in I slight tendency tion (0 76°) wit been found (Clark, 1962, vertical incre head tilt angl the head til 1861) Devia head tilt (M become less

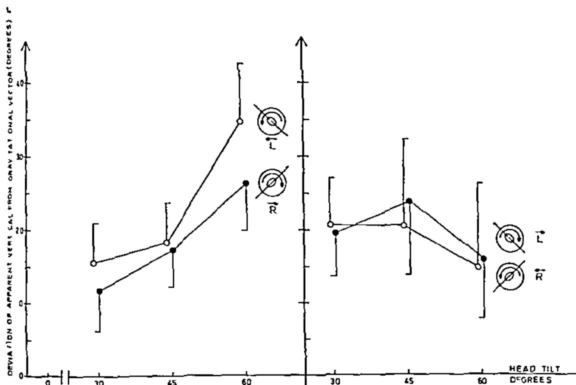


Fig 4 *Total tilt effects* ($\gamma = \alpha + \beta$) *with the visual stimulus rotating and the head at different angles of inclination* Disk rotation and head inclination towards the same direction (left graph) and in opposite direction (right graph) ● AV to the right ○ AV to the left

vertical According to Nagel (1898), Bourdon (1906) Muller (1916) and Bauermeister (1964) the Muller phenomenon persists until the head tilt angle exceeds 60° In the three subjects participating in this study we have not seen the Müller phenomenon when the head inclination reached 60° The accuracy of settings to AV with the head upright ranges within ±1 5°, but decreases with increasing head tilts

Tilt effects on apparent vertical induced by seen motion in different head positions (β)

The effects of a visual display rotating around the observer's line of sight increase with progressive inclination of the head The increase can be seen in Fig 3 (ordinate) It is represented by the deviation of the four curves from the dashed lines The latter depict the tilt effect induced by the moving display in the CW- and CCW direction, while the head was erect The curves represent the data obtained when the head was laterally inclined As can be seen from the picture, this increase depends on the directions of head tilt and display rotation The average effects are overtly smaller when the two stimuli point towards the same direction than vice versa This asymmetry cannot be explained as the result of summation of the "head" and "disk effect" since the data depicted in this graph have been obtained by subtracting the head tilt-effect (α) from the total effect (γ) shown in Fig 4 Fig 3 therefore depicts the net effect of the moving visual display in different head positions Standard deviations of 'head effects' (α) and 'visual effects" (β) are very similar as long as the two point towards the same direction, but increase markedly when the visual stimulus moves opposite to the head tilt As can be by comparing Fig 2 and 3 the additi[illegible] of the moving visual display (β)

the pure head tilt effect (α) Thus within the range of head inclinations explored the *orientation of AV was always determined by the direction* of the rotating visual display (Fig 4)

DISCUSSION

The data presented confirm the hypothesis that visual motion information and graviceptive information are centrally combined for the computation of the apparent orientation of gravity Our argument is based on the finding that the effect of a visual display rotating around the observer's line of sight increases with progressive lateral inclination of the head, positions in which statolithic information is known to decline in accuracy (Colenbrander, 1963/64, Graybiel & Clark, 1962, Graybiel & Patterson, 1955, Quix & Eijsvogel, 1929, Schone, 1964) and "sensory weight" as one *might like to call it* Our results seem to bear out that this decrease at least in part may be compensated by visual afference The data may to some extent be contaminated by the fact that our subjects were tested with the head bent with respect to the body therefore allowing for possible interfering afference from joint receptors and graviceptive information from somatoreceptors from the trunk which was kept in the upright position This interference, however, would only change the amount of induced tilt and is not conditional on the described phenomenon as was indicated by pilot experiments performed with the entire body tilted laterally Earlier studies comparing the effects of body versus head inclination and head versus body inclination on AV yielded controversial results Fischer (1927) *found in one* subject that the effects of head and trunk inclination are quite similar and add up to the equivalent of the effect of inclination of the entire body Witkin & Asch (1948), Schöne & Udo de Haes (1968) and Wade (1968), however, found little difference between exclusive head tilt and tilt of the entire body

The neurophysiological basis for this type of visual-vestibular interaction is not known An interaction of graviceptive and visual inputs in the vestibular nuclei, however, is [illegible] since related interactions have been sh[illegible] *animals with rotation in the horizontal* [illegible] about an earth vertical axis Optokinetic [illegible] mation was shown to influence the di[illegible] of the first order vestibular neurone in the [illegible] fish (Klinke & Schmidt, 1970) and of [illegible] vestibular nuclei in the goldfish (Dichgans et [illegible] 1973), rabbit (Dichgans & Brandt, 1972 [illegible] pold et al , 1973) and monkey (Henn et al, [illegible] mitted) The functional importance of [illegible] vestibular interaction during motion in [illegible] horizontal plane is rather obvious in that vestibular semicircular canals only provide [illegible] formation about changing velocities [illegible] the visual input contributes supplementary [illegible] formation about constant velocity (Brandt [illegible] 1973, Dichgans et al , 1973) With mo[illegible] about an axis that is perpendicular to [illegible] orientation *of gravity visual afferents may* [illegible] only interfere with canal input but also afference from statoliths and other grav[illegible] receptors The interaction might not at all [illegible] not exclusively occur at the level of the vest[illegible] lar nuclei but at higher levels of the central [illegible] vous system To clarify these mechanisms [illegible] challenging subject for further investigation

The functional importance of visual-vest[illegible] interaction during motion in a vertical plane [illegible] not as well understood at the present time [illegible] conceivable, however, that the effect of rel[illegible] motion of the visual environment caused by [illegible] body displacement from real upright [illegible] corroborate the actual information from gr[illegible] ceptors for postural adjustment On the [illegible] line one might give a functional interpreta[illegible] *for the asymmetry* in the amount of tilt of [illegible] induced by the moving visual display when [illegible] inclination and visual movement point to [illegible] the same direction as opposed to the si[illegible] when they are in opposite directions. [illegible] natural conditions body tilt causes r[illegible] motion of the visual scene that is oppos[illegible] direction to the body displacement The r[illegible] relative motion of the visual environment, [illegible] this case, will corroborate with the actual [illegible] formation from graviceptors for postural ad-

, whereas with the two motions going in the direction visual afferents would antagonize tural uprighting process induced by gravi e inputs The latter obviously represents tificial situation created in the laboratory s of no behavioural significance in normal A similar asymmetry has been found for fects of steadily tilted contours which also ronger when head tilt and contour tilt are posite directions (Mann, 1952, Witkin & 1948) It seems to us that the postural of stationary and moving scenes repre respectively, the static and dynamic con ons of vision to graviceptive orientation

ZUSAMMENFASSUNG

nehmender seitlicher Kopfneigung (0 , 30 , 45° sche nt d e subjektive Vertikale zunehmend zur Seite gene gL Gleichzeitig steigt die Varianz von ungen der visuellen Vertikalen an was durch nehmende Ungenauigkeit des Statolitheneinflusses licher Kopfneigung erklart werden kann Die enwahrnehmung wird auch durch großflachige

u Bestimmung der Schwerkraftorientierung bei mit zunehmender Kopfneigung der graviceptive weniger genau und gewichtig wird kann der Anteil entsprechend zunehmen Tatsachlich sind ell induzierten Auslenkungen der wahrgenomme tikalen bei aufrechtem Kopf geringer und nehmen ehmender Kopfneigung zu Die Potenzierung des uellen Einflusses auf die subjektive Vertikale bei igung ist am großten wenn der visuelle Bewe z in Gegenrichtung der Kopfneigung rotiert Die llte visuo-graviceptive Interaktion hat auch unter hen Bedingungen eine funktionelle Bedeutung Regulierung der Korperhaltung

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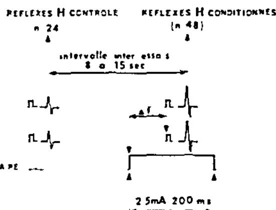


Fig 1 Organisation de la double stimulation stimulation vestibulaire (St V) stimulation de Hoffmann (H). Dans une série expérimentale, comportant 72 réflexes H la stimulation vestibulaire appliquée d un même côté (à gauche ou à droite) La stimulation réflexogène est appliquée simultanément sur les deux jambes. On distingue les réflexes H contrôle (n = 24 par série) des réflexes H conditionnés par la St. V (n = 48 par série) Commentaires dans le texte.

ficacite de la St V dépend du cote ou elle est appliquee (a gauche ou à droite) Par ailleurs, nous soulignons le role de la latéralisation motrice des sujets dans la reactivité reflexe spinale la facilitation des reflexes H depend du coté de la réception électromyographique (jambe gauche ou jambe droite)

Les variations differentielles des reflexes enregistres bilateralement a la suite d une St V monaurale sont donc affectées

1 Par l organisation asymétrique des voies vestibulo-spinales issues d un labyrinthe (prepondérance des voies ipsilaterales)

2 Par la sensibilite differentielle des récepteurs labyrinthiques symétriques à la stimulation electrique (prevalence vestibulaire)

3 Par la reactivite différentielle des noyaux moteurs spinaux symetriques (latéralisation motrice)

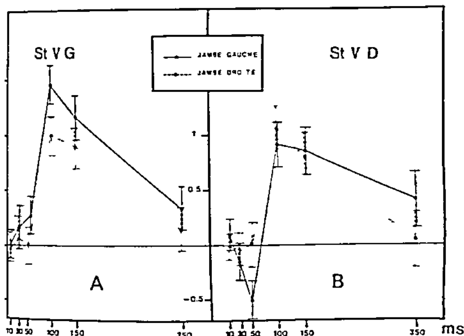
METHODES ET TECHNIQUES

Vingt sujets normaux, etudiants de 18 à 25 ans, remunérés et 2 sujets delabyrinthes ont éte examinés Pour l étude des effets spinaux de la St V, on enregistre le réflexe H dans un muscle extenseur du pied le soléaire le sujet est assis dans un fauteuil specialement equipé

La stimulation réflexogène est une stimulation électrique percutanée du nerf sciatique poplité interne, appliquée selon la méthode définie par Paillard (1955) elle est distribuée simultanément dans les deux jambes On recueille la réponse reflexe H par électrodes de surface et mesure son amplitude au voltmètre électronique Nous déterminons pour chacun des sujets l amplitude maximale des reflexes H à droite et à gauche et nous réglons l intensité de la stimulation reflexogene de façon à obtenir pour chaque jambe un reflexe d amplitude sous-maximale

La St V est une stimulation électrique percutanee de la region otique Elle consiste en un stimulus rectangulaire de 200 ms appliqué au moyen d un casque leger supportant deux électrodes d argent une anode de 2 cm de diamètre est appliquée en avant du tragus, et la cathode de 1 cm de diamètre sur l'apophyse mastoïde située du meme côté de la tête La St V provoque chez le sujet assis des inclinaisons de la tête, dans les conditions de l'expérience elle est ajustée à une intensité subliminaire pour ces déplacements (soit 2,5 mA en moyenne)

Chaque sujet est soumis à 4 séries expérimentales reflexologiques 2 avec la stimulation vestibulaire appliquée au côté gauche (St V G) et 2 avec la stimulation vestibulaire appliquée au côté droit (St V D) Chaque série (Fig 1) comporte 72 réflexes H dont 24 ne sont pas conditionnés par la St V et constituent la distribution contrôle Les reflexes H conditionnés sont provoqués à des délais Δt variables (10, 30, 50, 100 150 et 350 ms) distribués de façon



Modulation de l'amplitude des reponses reflexes d tiennes par la stimulation vestibulaire electri (St V) Resultats moyens observes sur une popula e 20 sujets (A) Effets spinaux de la St. V appli u cote gauche (St V G) (B) Effets spinaux de V appliquee au cote droit (St V D) *En abscisses* en millisecondes entre le debut de la St. V et la ation reflexogene *En ordonnees* amplitude des es H exprimee en valeurs reduites (notes Z) Les variations d amplitude des reflexes H recueillis sur la jambe gauche (trait plein) et sur la jambe droite (trait pointille) sont fonction du cote d'application de la St. V On remarque que si les effets contralateraux sont identiques, les effets ipsilateraux sont differents (meilleure efficacite de la St. V G) Chaque point des courbes re presente la moyenne de 320 reflexes limites de confiance a 0 05

ire La duree de l intervalle inter-essais au hasard entre 8 et 15 secondes Les es H controles et les reflexes H condition sont provoques simultanement dans les jambes leur présentation suit un ordre lo-aleatoire

s experiences complementaires ont ete ees sur des sujets normaux en plaçant les odes de la St V sur la joue Les sujets soumis aux memes series experimentales stimulation cutanee est identique en duree ensite a la St V Cette situation controle itue une precaution d ordre methodologi elle a pour but essentiel parallelement aux iences realisees sur des sujets delabyrin de discuter la specificite de la St V et de er l importance des influences aspecifiques gine cutanee liees a la stimulation electri e la region otique

Par ailleurs nous avons employe des tests cliniques classiques pour determiner la lateralisation motrice des sujets au niveau des membres superieurs et des membres inferieurs L epreuve de la marche aveugle a ete utilisee comme test de la dominance vestibulaire apres avoir repere une cible placee a 4 metres devant eux, les sujets ont pour consigne de marcher vers la cible les yeux bandes Nous apprecions les deviations a droite ou a gauche dans la trajectoire suivie

Le traitement des donnees a consiste en operations de calcul de la moyenne et de l ecart type des valeurs controles l amplitude des reflexes H conditionnes a ete exprimee en pourcentage de la reference ou en notes reduites Z (c est a-dire en ecarts-types de la distribution des valeurs controles) Les calculs sont effectues par sujet, en fonction du cote de la recep

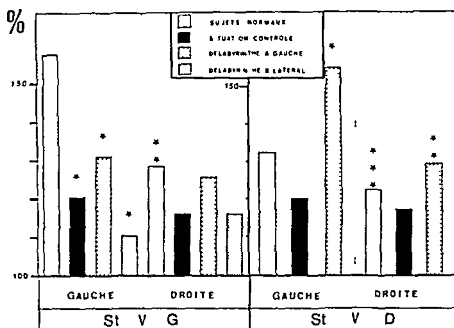


Fig 3 Diagramme des facilitations des réponses réflexes H conditionnées Chez les sujets normaux ($n = 20$) chez le sujet délabyrinthé bilatéral et chez le sujet délabyrinthé à gauche les réponses réflexes H sont conditionnées par la stimulation vestibulaire En situation controle, les réflexes sont conditionnés par une stimulation électrique de la peau Nous comparons la facilitation des réflexes obtenue 100 ms après le début de la stimulation (cutanée ou vestibulaire) L amplitude des réflexes est exprimée en pourcentage de la référence (réflexes non conditionnés) On distingue 4 effets qui correspondent aux 4 cases de la figure l effet ipsilatéral et l'effet controlatéral de la stimulation vestibulaire appliquée au côté gauche (*St V G*) l effet ipsilatéral et l'effet controlatéral de la stimulation vestibulaire appliquée au côté droit (*St V D*) * indique une différence significative ($P < 0\ 05$) dans chaque cas, par rapport aux sujets normaux ** indique une différence significative ($P < 0\ 05$) entre l effet ipsilatéral et l effet controlatéral de la St V *** indique une différence significative ($P < 0\ 05$) entre l effet ipsilatéral de la St V G et l'effet ipsilatéral de la St V D

électromyographique, du coté de la St V et du délai interchocs Δt Le programme de traitement des données reprend ces diverses opérations pour l ensemble des sujets

RESULTATS

1) *Modulation de l amplitude des réflexes H conditionnés*

a) Chez les sujets normaux

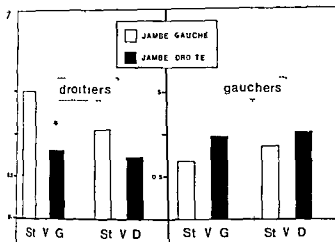
Le décours des variations moyennes de l amplitude des réflexes H conditionnés par la St V est décrit par la figure 2, il diffère selon que la St V est appliquée a gauche ou à droite

— en St V G (Fig 2A) un effet facilitateur ipsilateral se manifeste dès 50 ms et culmine a 100 ms ou il représente environ 160 de la référence (écart de 1 5 écart ... latéralement, la facilitation ... 100 ms est significativement

— en St V D (Fig ... latérale représente 130 ms (écart de 1 ... se développe une a 50 ms, suivie identique a la

Quel que soit V , la facilitatic mais elle reste d excitabilité c

Les réfl facilitation sent une f de la St V la St V ...



¬ 4 Lateral sation motrice et facilitation vestibulo spinale Comparaison de la facilitation moyenne des ... conditionnés par la St V au delai 100 ms chez gauchers ($n=4$) et chez des droitiers ($n=12$) Les ats sont exprimes en écarts types de la distribution valeurs contrôles (notes Z) La facilitation des reflexes est plus importante dans la jambe non préferentielle quel que soit le coté d application de la St V (a gauche St V G ou à droite St V D) * indique une difference significative ($P<0\,05$) entre l amplitude des reflexes recueillis sur la jambe gauche et l amplitude des reflexes recueillis sur la jambe droite

une inhibition précoce par la St V appli ... au coté droit

Situation controle

Les reflexes H conditionnes par une stimulation ectrique cutanee de la region des joues (cf ıques) sont facilites (Fig 3) La facilitation ...istree est significativement moins impor ante que celle provoquee par la St V et ne ı pas du cote de la stimulation cutanee gauche ou joue droite) Elle est maximale delai 100 ms ou elle represente 120 % de la en ce qui concerne les reflexes à gauche et 115 % pour les reflexes a droite (dif ference non significative)

En situation controle nous n observons ja mais d effets precoces facilitateurs ou inhibi teurs

:) *Chez les sujets delabyrinthes*

Deux sujets ont ete examines L un avait subi une neurectomie vestibulaire chirurgicale therapeutique a gauche le second est classé cliniquement d elabyrinthe bilatéral par barotraumatismes (destruction des organes sensoriels) Les resultats sont reportes dans la figure 3

La facilitation des reflexes H chez le sujet delabyrinthe bilateral est identique a celle obtenue en situation controle

La comparaison des facilitations chez le de labyrinthé a gauche et chez les sujets normaux montre des differences importantes Chez le delabyrinthe a gauche, la St V appliquée a gauche (cote lese) est moins efficace et produit des facilitations ipsilaterale et contralaterale moderees et symetriques Appliquee a droite (cote sain) elle se revèle plus efficace et produit des effets ipsilatéraux et contralateraux asymetri ques la facilitation des reflexes H a gauche est significativement plus grande que chez les sujets normaux On peut parler d hyperexcitabilité vestibulaire pour le côte intact et d hypoexci tabilite pour le cote lése

En resumé La facilitation maximale des reflexes, obtenue au delai 100 ms est asymetri que elle est fonction du cote d application de la St V et est plus importante en moyenne a la suite d une stimulation electrique du laby rinthe gauche Nous faisons l hypothèse d une prevalence vestibulaire gauche Par ailleurs quel que soit le cote d application de la St V, la facilitation est en moyenne plus grande ... reflexes recueillis dans la jambe gau... effet se manifeste egalement en ...

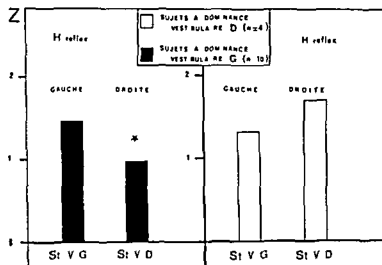


Fig 5 Prévalence vestibulaire et facilitation vestibulo-spinale Comparaison de la facilitation moyenne des réflexes *H* conditionnés par la stimulation vestibulaire au délai 100 ms chez les sujets à dominance vestibulaire droite (en noir) et chez les sujets à dominance gauche (en blanc) Les résultats sont exprimés en écarts types de la distribution des valeurs contrôles (notes Z). Seuls les effets ipsilatéraux sont représentés On observe que la facilitation des réflexes est *toujours plus importante pour* la stimulation électrique du labyrinthe dominant * indique une différence significative ($P<0\,05$), entre les facilitations de la St V G et la St V D

trôle (cf Fig 3) On peut penser a une manifestation de la lateralisation des sujets compte tenu que la *majorite d'entre eux ont la même* dominance motrice droite La participation de deux facteurs (prévalence vestibulaire et latéralisation motrice) dans la définition de l effet vestibulo spinal asymetrique consécutif a une stimulation vestibulaire unilaterale, a éte étudiée en regroupant les sujets normaux en fonction de leur latéralisation *motrice, puis en* fonction de leur dominance vestibulaire observée au plan clinique

2) *Lateralisation motrice et facilitation vestibulo-spinale*

A l issue des tests cliniques de latéralisation (cf techniques), 4 gauchers et 12 droitiers *ont été* retenus Quatre sujets dont la dominance motrice n était pas nette ont éte éliminés

On constate l existence d une relation entre *l effet facilitateur de la St V* sur les réflexes H et la latéralisation la facilitation la plus importante est enregistree sur la jambe non préférentielle (Fig 4) La différence entre la facilitation des reflexes H a gauche et les reflexes H à droite est significative ... les droitiers, une tendance identique est notée chez les gauchers Ces résultats sont en accord avec *les données classiques* qui considèrent que droitiers et gauchers n ont pas une organisation motrice en miroir

La plus grande reactivité réflexe de la jambe non préferentielle, quel que soit le côté d'application de la St V, nous amène à considérer le facteur latéralisation comme facteur indépen*dant, responsable pour* une part de l asymétrie des effets vestibulo-spinaux

3) *Prevalence vestibulaire et facilitation vestibulo-spinale*

Les sujets sont regroupés selon leur dominance vestibulaire testée par l épreuve de la marche aveugle (cf techniques) En accord avec la clinique nous avons interprété une déviation de la trajectoire vers la droite comme le signe d'une dominance vestibulaire gauche et vice versa Quatre sujets a dominance *droite* et 10 sujets à dominance gauche ont eté retenus

Une corrélation étroite est établie entre l'asymétrie des fonctions vestibulaires observée au plan clinique et la plus ou moins grande efficacite de la St V selon qu elle est appliquée au

côté fonctionnellement dominant ou au côte fonctionnellement dominé : la stimulation électrique du labyrinthe dominant provoque une facilitation des reflexes H ipsilatéraux plus importante que celle évoquée par la stimulation du labyrinthe dominé (Fig 5)

Chez les sujets classés à dominance vestibulaire gauche, la facilitation obtenue, par stimulation du labyrinthe gauche, sur la jambe gauche, est significativement plus grande que la facilitation obtenue par stimulation du labyrinthe droit sur la jambe droite

Chez les sujets classés a dominance vestibulaire droite on observe, en fonction de la dominance, une difference des facilitations ipsilatérales qui va dans le même sens sans toutefois etre significative

Cette sensibilité differentielle des structures labyrinthiques a la stimulation électrique contribue à définir, independamment de la lateralisation motrice, des effets spinaux asymétriques

DISCUSSION

1) *Effet spinal de la stimulation electrique et structures nerveuses centrales impliquees*

Le recours à la pathologie humaine du labyrinthe nous permet de préciser le point d impact de la St V Une lésion du système vestibulaire peripherique (recepteurs sensoriels) rend dans nos conditions la St V inefficace (cas du sujet délabyrinthe bilatéral) Une section complete de la branche vestibulaire du VIII (cas du sujet de labyrinthé à gauche) reduit considérablement l'efficacite de la St V appliquée au côte lésé Nous avons recemment entrepris des neurectomies vestibulaires unilatérales chez le Singe (Papio papio) Les resultats préliminaires obtenus en employant les techniques utilisees chez l Homme et les memes parametres de stimulation, indiquent que la St V appliquée au côte lese n'influence pas l'excitabilité reflexe spinale par contre les réflexes H sont normalement facilités lorsque la St V est appliquée au côté sain Pour des intensités élevees (de l'ordre de 6 à 8 mA), la stimulation du côté lésé retrouve son efficacité et provoque une modulation de l'excitabilité spinale

Ces résultats nous permettent de conclure au point d'impact périphérique et sensoriel de la St V pour les intensités de stimulation employées chez l'Homme (2,5 mA), et a une atteinte moins sélective du système vestibulaire pour des intensités élevées (excitation directe du nerf vestibulaire) L'hyperexcitabilite observee par stimulation du côté sain chez le sujet délabyrinthe unilatéral paraît liée au développement d'un mécanisme central de compensation Selon Pfaltz & Piffko (1972), un mécanisme possible de la compensation serait basé sur la modulation de la réactivité de l'organe sensoriel intact au moyen de fibres efferentes Cette hyperexcitabilité existe chez le Singe dans les 3 ou 4 jours qui suivent l'intervention chirurgicale

L'effet spinal de la St V consiste essentiellement en un relèvement de l'amplitude des reflexes H C'est une facilitation breve mais importante de la réactivité reflexe spinale, principalement ipsilatérale, qui culmine a 100 ms ou elle représente au maximum 160 % de la reference Dans nos conditions expérimentales, cela correspond à un recrutement d'environ 15 % de la frange motoneuronale activee subliminairement par la volée afférente réflexogène Ia La comparaison des resultats obtenus en situation contrôle, chez les sujets normaux et dans la pathologie, nous renseigne sur la nature de l'effet spinal de la St V La facilitation des reflexes H, 100 a 150 ms après le début de la St V, resulterait de la combinaison de deux influences mises en jeu par la stimulation électrique par voie externe l'une, specifique, correspondrait à l activation des récepteurs sensoriels labyrinthiques, l autre, aspecifique, serait liee à la stimulation électrique de la peau

Nous interpretons la facilitation des réflexes en situation contrôle et chez le suje rinthé bilatéral comme le signe d'u réticulaire aspecifique à point de Cette composante reticulaire des reflexes a eté souvent

par Paillard (1955) qui étudiait la modulation du reflexe H conditionné par un son, il concluait a une action sur les motoneurones spinaux par les mêmes circuits que ceux qui organisent la réaction de sursaut

Les voies nerveuses empruntées par les influences spécifiques d'origine vestibulaire ne sont pas précisées par cette étude Le délai necessaire a l activation des structures labyrinthiques par la St V n'est pas connu, nous ne pouvons qu'émettre des hypotheses sur le problème de l organisation des liaisons vestibulo-spinales chez l Homme

Le faisceau vestibulo spinal latéral, originaire du noyau de Deiters ipsilatéral atteint les regions lombaires il peut constituer une voie d expression des influences vestibulaires spécifiques L'existence de projections vestibulo-réticulaires suggere que les voies suivies pourraient être vestibulo-réticulo-spinales Ce décours réticulaire expliquerait pour une part l effet contralatéral de la St V

L'intervention du cervelet est à considérer des fibres vestibulaires primaires projettent directement sur le lobe flocculo-nodulaire Les travaux de Brodal (1960) soulignent la richesse des connexions entre le système vestibulaire et le cervelet et montrent, au moins chez le Chat, l existence d un système d afférences bien organisé Schématiquement, on distingue deux ensembles fonctionnels sur chaque noyau vestibulaire l un recevant des afférences vestibulaires primaires, l autre ou convergent des influences en provenance du noyau fastigial et du cortex cérébelleux (pour le noyau de Deiters, cette dernière région est en relation avec les noyaux moteurs des membres postérieurs) Les connexions sont principalement ipsilatérales A partir du noyau fastigial, elles devraient etre facilitatrices, à partir de l archéocervelet inhibitrices On comprendrait alors que des effets opposés puissent s'exprimer aux delais précoces 30 et 50 ms et la facilitation des reflexes H, 100 a 150 ms après le debut de la St V represen terait le rebond classique de l'inhibition consécutif a une activation cérébelleuse

2) *Rôle de la latéralisation motrice*

Les travaux de réflexologie consi[illegible] habituellement que la réactivité réflexe au niveau des membres postérieurs est identique L étude des courbes de recrutement du réflexe H et des courbes d excitabilité effectuées à droite et à gauche ne montre pas de difference significative Par contre, les effets spinaux de la St [illegible] dépendent en partie de la latéralisation des sujets quel que soit le côté d application de la St V, la facilitation du réflexe H est plus importante en moyenne pour la jambe non préférentielle

Nous pensons que l'asymétrie de la réaction spinale exprime la dominance hémisphérique Chez l Homme en effet, la qualite des performances motrices que réalisent chacun des membres est inégale, en particulier, les membres postérieurs ont un rôle fonctionnel [illegible] dans l ensemble des habitudes motrices et des gestes automatisés du comportement locomoteur Les differences fonctionnelles [illegible] font supposer que les contrôles supraspinaux qui s'appliquent à des noyaux moteurs [illegible] ques n'ont pas la même efficacité Ces contrôles [illegible] seraient plus importants sur l hémicorps [illegible] d un droitier, ils détermineraient la [illegible] des motoneurones aux influences per[illegible] et centrales

Nous avons recemment repris [illegible] de l'asymétrie de la réactivité réflex[illegible] postérieurs (Bonnet & Lacour, [illegible] portait sur les effets d un son b[illegible] les réflexes de Hoffmann, da[illegible] composée de droitiers et de ga[illegible] sélectionnes Une facilitation [illegible] H est observée, interprétée [illegible] tion réticulaire On const[illegible] asymétrique la facilitat[illegible] significativement plus im[illegible] non préférentielle et la [illegible] est plus faible

Les résultats qu'appo[illegible] lité sont interpretables [illegible] l activité des structures [illegible] sait que la variabilité d[illegible] système composé d u[illegible]

est d autant plus faible qu'il y a independance entre les elements qui le composent L'introduction d une correlation, même faible, entre plusieurs de ces eléments (par exemple l action d'une cause commune), accroît la variabilite de la reponse globale du système On peut concevoir que toute manifestation de contrôle de l'activité du noyau moteur par les structures centrales, qui tend a faire correler l activite des motoneurones, a pour resultat d'augmenter la variabilite de la reponse globale En termes plus physiologiques, on pourrait dire que l importance de la variabilite des reponses reflexes sur la jambe preferentielle, traduirait l'existence de facteurs corrélants du niveau d'excitabilite des motoneurones, c'est a dire l existence de contrôles centraux plus importants et efficaces que sur la jambe non preferentielle

On comprendrait alors l existence d'une plus grande reactivite a la St V des reflexes H de la jambe non preferentielle, leur plus grande bilité aux influences vestibulaires Le sup- anatomo-fonctionnel de ce controle cortical differentiel sur des structures spinales symetri- serait celui de l asymetrie pyramidale, genetiquement determinee pour une part, et renfor- par les processus d apprentissage

) La prevalence vestibulaire

Chez la majorité des sujets le labyrinthe gauche st plus réactif a la stimulation electrique la litation du reflexe H est significativement uperieure en moyenne lorsque l on stimule le labyrinthe gauche Ce resultat ne parait pas dependre de la lateralisation motrice des sujets si l on regroupe les sujets selon leur dominance vestibulaire testée par l epreuve de la marche aveugle, on constate (Fig 5) que la facilitation des reflexes ipsilateraux est plus importante lors de la stimulation du labyrinthe dominant La forte proportion, dans notre echantillon, de sujets a dominance vestibulaire gauche explique l observation globale d une prévalence du systeme vestibulaire gauche

Dans leur etude des deviations laterales de l axe corporel provoquees par une stimulation galvanique du labyr... (1972) concluent également a une prédominance du système vestibulaire gauche Ils notent un nombre significatif d'inversions de la direction des oscillations laterales du corps lorsqu'ils inversent la polarite de la stimulation du labyrinthe gauche la polarisation anodique ou cathodique du labyrinthe droit n'affecte pas le sens des deviations Les auteurs comparent ensuite la direction de la rotation qui provoque une reponse nystagmique de plus haute frequence a la direction de la déviation de l axe corporel provoquee par leur stimulation galvanique, ils montrent une correlation positive entre la rotation horaire (a droite) et la stimulation cathodique du labyrinthe gauche (stimulation qui evoque une deviation vers la droite) Ils n'observent pas de correlation entre le sens de la déviation et la lateralisation motrice des sujets D autres travaux (Milojevic & Watson, 1965), relatifs a la reponse nystagmique au test calorique, indiquent une « preponderance directionnelle » pour la stimulation du labyrinthe gauche Cette reponse nystagmique plus importante a la stimulation calorique (44°) de l'oreille gauche se manifeste chez des droitiers et chez des gauchers De la même façon, notre etude n etablit pas de relation nette entre la prevalence vestibulaire et la latéralisation, mais une tendance se manifeste on rencontre plus fréquemment l association (dominance motrice droite — prevalence vestibulaire gauche)

Deux questions se posent au sujet de la prevalence vestibulaire

quels mecanismes peuvent rendre compte de la sensibilite differentielle des recepteurs labyrinthiques a la stimulation électrique ?

Quelle serait la signification fonctionnelle d une prevalence vestibulaire ?

La reponse a la premiere question peut s appuyer sur les donnees de Gernandt (1967) Au plan anatomique, l existence d'un faisceau de fibres efferentes a éte démontree au niveau des récepteurs labyrinthiques au plan fonctionnel, ce contingent efferent serait capable de régler la sensibilite des recepteurs Un tel controle efferent se retrouve en effet dans la majorite des

bulaire, il serait essentiellement réalisé par l'intermédiaire de fibres non croisées atteignant les récepteurs et les noyaux vestibulaires Son origine corticale ou sous-corticale est controversée, son action inhibitrice probable Nous faisons l'hypothese qu'une intervention différentielle de ce contrôle sur les structures vestibulaires symétriques est a l'origine de la différence de réactivité constatée Par ailleurs, on peut envisager que la réponse sensorielle labyrinthique est identique de chaque côté mais qu'elle s'exprime a travers des structures latéralisées La richesse des connexions vestibulo cérebelleuses suggère qu'il existe peut être une relation entre asymétrie vestibulo-spinale et asymétrie cerebelleuse L'emploi de tests cliniques purement cérébelleux serait de ce point de vue à envisager

L'action différentielle d'un contrôle cortical (ou sous-cortical) sur des structures receptrices sensorielles symétriques est une hypothese plus séduisante Si elle se trouvait confirmée, elle entrerait dans le cadre des données relatives à la sensibilité differentielle des recepteurs pairs Un certain nombre d'asymetries auditives ont été [illegible] en evidence chez l'Homme, en particulier [illegible] avantage perceptif de l'oreille gauche pour les [illegible]lus non verbaux (Botte et Chocholle, 1972) De la meme façon, on constate l'existence d'une dominance oculaire Il semble que dans chaque couple que constituent les systèmes sensoriels symétriques, se manifeste une asymétrie fonctionnelle La prévalence observée pour le système vestibulaire pourrait relever d'une organisation analogue

SUMMARY

The organization of postural reflex reactions following labyrinthine excitation was taken up in man by studying interactions between descending vestibular influences and spinal activities involved in segmental proprioceptive reflexes. 20 normal and 2 abnormal subjects were examined they were submitted to a monaural galvanic vestibular stimulation checked by the H reflex amplitude and to both motor and vestibular testing The results indicated an asymmetrical increase in the ipsi and contralateral H reflex variations depending on the essentially homo ipsilateral vestibulo-spinal connections on a more important reflex reactivity in the non preferred leg and on a unilateral vestibular prevalence Vestibular prevalence and asymmetrical reflex reactivity in both legs was discussed referring to a differential central control over symmetrical effector and receptor structures

ZUSAMMENFASSUNG

Die Autoren untersuchen die Beeinflussung der Erregbarkeit spinaler Reflexschaltkreise (monosynaptischer Reflex nach Hoffmann) durch monaurale bipolare elektrische Vestibularreizung Diese Reizung provoziert eine Erhöhung der Amplitude des H Reflexes Auf der [illegible] Seite bewirkt sie im Mittel eine Bahnung signifikant grösseren Ausmasses Die Hypothese einer vestibulären Dominanz wird diskutiert Dieses Ergebnis hängt eng mit der Richtungsabweichung von Versuchspersonen im Gehversuch mit verbundenen Augen zusammen Die Analyse der Ergebnisse weist ausserdem auf unterschiedliche Reaktivität in beiden Beinen hin Die Bahnung der H Reflexe ist im nicht dominanten Bein immer bedeutender Die Begriffe der vestibulären Dominanz und der Asymmetrie der Reflexreaktivität in den unteren Extremitäten werden im Rahmen einer differenzierten Kontrolle auf die zentralen Ursachen und symmetrische motorische und sensorische Strukturen hin diskutiert

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SUBTOTAL EVULSION OF THE FACIAL NERVE IN HEMIFACIAL SPASM

J Sade

From the Meir Hospital, Kfar Saba, Israel

(Received February 19, 1974)

Abstract The various surgical procedures performed for hemifacial spasm whereby part of the facial nerve is sectioned indicate (1) An intermingled intrafacial arrangement of the various nerve filaments This arrangement differs from the classical concept that there is a specific regional representation of the peripheral branches and of their tracts in the main trunk The intermingling of all peripheral filaments in the main trunk explains why even a small portion of the main trunk may represent functionally most of the face (2) The presence of a wide peripheral anastomosing network connecting the peripheral branches in such a manner that one branch may functionally represent the other branches if they are severed

On the basis of these anatomical observations and because of the frequent recurrence of facial spasm after most prescribed surgical procedures evulsion of most peripheral twigs is recommended in certain cases The mandibular branch and one or two superior branches are left This is considered a compromise surgical procedure, resulting in only slight facial weakness as well as possible recurrence of mild and relatively sparse spasmodic movements

Further theoretical conclusions from the intrafacial fascile arrangements constitute an *a priori* limitation of surgical results from facial nerve anastomosis and grafting in traumatic cases

Hemifacial spasm is a disease characterized by involuntary one-sided tonic- and clonic facial movements In the early stages the spasm occurs at infrequent intervals, mostly around the orbicularis oculi However, in time most of the facial muscles often become involved (Fig 1) the spasm recurs at more frequent periods—up to many times a day—and has the appearance of a facial "mass movement", thus constituting a formidable nuisance The disease is usually seen in adulthood and should be differentiated from nervous tics, post traumatic tics or spasms secondary to a central nervous lesion True hem[illegible] facial spasm can also appear during sleep (Green[illegible] wood, 1946, Potter, 1972) and cannot be stopped voluntarily, whereas a nervous tic does not appear during sleep, can be controlled to some degree, and is usually not confined to the facial nerve Traumatic tics (Fowler, 1939) have by definition a traumatic history, while those secondary to central nervous lesions are [illegible] tonic in character and are associated with other central nervous symptoms Hemifacial spasm has been well reviewed by Ehni & Woltman (1945) and O'Donnell (1953)

The number of treatments suggested for this disease are many, as is the case in diseases for which there is no really satisfactory treatment Tranquilizers, muscle relaxants and [illegible] therapy have not been found helpful (Green[illegible] wood, 1945, McCabe 1970) Dividing the facial nerve and anastomosing it to the hypoglossal or spinal accessory was found to stop the spasm (Ehni & Woltman, 1945) but the loss of [illegible] factory facial control by the patient makes the method unacceptable Rhizotomy was also tried and abandoned (Ehni & Woltman, 1945)

Injection of denaturating compounds such as phenol or alcohol (Greenwood, 1946, Wakasugi, 1972) into the peripheral branches of the nerve or into its main trunk is rather popular because of the technical simplicity of the method and initial effectiveness, however the beneficial effect is only temporary and recurrence is the rule (McCabe, 1970, Wakasugi, 1972) Subsequent injections are often less successful [illegible]



I Hemifacial spasm Patient showing involuntary of the right s de of her face—note th s move resembles a "mass movement

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In various nerve resection surgical methods ks were directed both at the main stem the peripheral branches Total division or ush ng of the main stem was practised by awthorne (1956) who reported his patients re free from spasms as long as the face was aralysed but that the spasm recurred once the regenerated Opinions vary as to which tuation is the more desirable—some prefer the rather than the paralysis and others vice Interestingly relief from spasm was also ter facial nerve decompression More ph st cated and interesting measures were roduced by German (1942) who sectioned agitud nally parts of the various peripheral (Fig 2)—this technique was later fol wed by Greenwood (1946) Miehlke (1959) and Diamant et al (1967) The initi l rest lts were satisfactory but the rate of reci rrence ofter necessitated further operations whict were not always easy to perform because of scarring

Scoville (1955) advocated a varint on of this procedure thinning out the ma i ficial stem (Fig 2) he cuts down to at least 2/3 of its depth to avoid a high recurrence rate Already in 1946 Greenwood advocated selective resection of some of the peripheral branches this procedure wa later taken up by Fisch (1972) though Boyl (1972) maintained that the branches shou n merely be cut but actually evulsed

McCabe (1970) pointed out th it all the a ve mentioned methods invariably end in recu re e of the spasms and advo ated therefore evulsion of the nerve—but for i s iandib cervical branches Such an operati r actually to an upper fac a paral eye and upper lip) ard hi e its as the spasm is concerned p obab the cost might seem to he at ier h

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SURGICAL METHODS AND PROCEDURES

Subtotal facial nerve evulsion—rationale and procedure

We are ignorant of the reason for the initial therapeutic benefit provided by the various facial resection and dilution techniques (chemical or surgical)—as well as to why the recurrence of spasms However one observation is that the spasmod c recurrences are often (though not

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Injection of denaturating compounds such as phenol or alcohol (Greenwood, 1946, Wakasugi 1972) into the peripheral branches of the nerve or into its main trunk is rather popular because of the technical simplicity of the method and initial effectiveness, however the beneficial effect is only temporary and recurrence is the rule (McCabe, 1970, Wakasugi, 1972) Subsequent injections are often less successful, possibly due



Fig 1 Hemifacial spasm Patient showing involuntary movement of the right side of her face—note, this movement resembles a "mass movement"

to a perineural fibrosing reaction which also makes subsequent surgery rather difficult (Sadé, 1972)

In various nerve resection surgical methods, attacks were directed both at the main stem and the peripheral branches Total division or crushing of the main stem was practised by Cawthorne (1956) who reported his patients were free from spasms as long as the face was paralysed, but that the spasm recurred once the nerve regenerated Opinions vary as to which situation is the more desirable—some prefer the spasms rather than the paralysis and others vice versa Interestingly relief from spasm was also reported after facial nerve decompression More sophisticated and interesting measures were introduced by German (1942) who sectioned longitudinally parts of the various peripheral branches (Fig 2)—this technique was later followed by Greenwood (1946), Miehlke (1959) and Diamant et al (1967) The initial results were satisfactory but the rate of recurrence often necessitated further operations which were not always easy to perform because of scarring

Scoville (1955) advocated a variation of this procedure, thinning out the main facial stem (Fig 2), he cuts down to at least 2/3 of its depth, to avoid a high recurrence rate Already in 1946, Greenwood advocated selective resection of some of the peripheral branches, this procedure was later taken up by Fisch (1972), though Boyle (1972) maintained that the branches should not merely be cut but actually evulsed

McCabe (1970) pointed out that all the abovementioned methods invariably end in recurrence of the spasms, and advocated therefore total evulsion of the nerve—but for its mandibular and cervical branches Such an operation amounts actually to an upper facial paralysis (forehead, eye and upper lip), and while its result as far as the spasm is concerned is probably very good, the cost might seem to be rather high

We have treated patients in our Department by phenol injections as well as various partial resections of the facial nerve branches (both partial longitudinal resection and selective whole branch amputation) Initial amelioration of the condition was followed usually by a recurrence Therefore, I looked for a method of providing longer lasting relief without having to resort to "above lower lip" facial paralysis Such a method is reported here as well as what can be learned from the various facial nerve surgical procedures with regard to the intimate axonal intrafacial neuroanatomical arrangements

SURGICAL METHODS AND PROCEDURES

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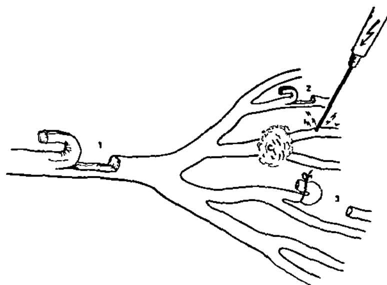


Fig 2 Various surgical treatments formed for hemifacial spasm (1) Thinn the main stem (Scoville) (2) Longit splitting of the peripheral branches (man, Greenwood Miehlke D et al) (3) Selective cutting of peripher branches (Greenwood Fisch) *C*, cotton with cocain to block retrograd electric impulses when the extent of stim ulus is tested

always) more or less in proportion to the amount of nerve tissue sacrificed initially

Clearly, the recurrent spasm after some peripheral branches are sectioned (Fig 2) must be through an efficient peripheral anastomosing network, which we do not usually see when exposing the facial nerve in the parotis (Fig 3) The notion that recurrence is due to regrowth f the nerves can be dismissed because we bend the stumps backwards (Fig 2) Furthermore the fact that no *selective* regional facial paralysis occurs after removal of several peripheral facial nerve branches would indicate that the remaining branches take over for those which have been sectioned, which would only be possible through a rather extensive peripheral anastomosing network Further evidence as to the existence of such a network (at least in cases of facial spasm) can be found on electrical stimulation of selective peripheral branches In this case, we have observed mass movement of all, or most of, the face, and not only muscle contraction of the narrow sections to which the particular branch is leading The possibility that such stimulation reached the other branches *retroactively* was eliminated by cocainization of the "hind" part of the nerve, thereby blocking retrograde impulses (Fig 2) We obtained the same result, indeed, also after the connection of that particular branch was severed from the main stem

Our surgical results and the rate of recurren should therefore related not only to th number of twigs resected but also to the re maining anastomosing peripheral network Bet results are achieved therefore if we *evulse* th nerve branches, as advocated by Boyle (1972).

The operation is done through a parotidec tomy skin incision and a rather full exposure o the facial nerve—its main trunk and the peri pheral nerve fibres (Fig 3*A* and *B*) No parotid gland tissue is sacrificed, however The operation consists of The pulling out, extirpation or evulsion of the individual facial branches, in stead of resecting them, rolling them on a hemostat "a la spaghetti' as much as possible before pulling them out—and thereby removing a nerve which is several centimetres long, in the hope that many of the peripheral anatomosing branches and their connections will be pulled out or severed In this fashion, all the periphera facial branches are eradicated except (1) The cervical branch—which usually plays an un important role in the spasmodic syndrome, (2 the mandibular branch which usually also plays only a relatively minor part in the spasmodic syndrome and which apparently has a poor (or no) anastomosing relationship with the other nerve fibres—as evidenced also by its weakness when it is inadvertently injured while perform ing a submandibular gland extirpation, (3



Fig 3A Exposure of facial nerve in the parot s *M* ma n stem at d v d ng po nt *X* small arrows sho v branches o be evulsed Large arrows ind cate branches left intact



Fig 3B Same pat ent as 3*A* after evuls on of branches S lk t es are seen as markers at po nts where the nerves were cut

one or two of the uppermost twigs (Figs 3*A* and *B*) It is this latter sole twig (or two) which will take over later adequate facial closure of he eye some movement of the upper l p and preservation of facial tone However it will also be responsible for the possible recurrence of some weak spasmodic episodes constituting the compromise —a fair price for the avoid ance of upper facial paralysis Whether to leave one or two twigs depends on their thickness—some information might also be provided through the nerve stimulator but essentially the decision w ll depend on the experience and judgement of the surgeon

The facial nerve stimulator is not absolutely necessary for the performance of the operation though it is a comfort to have such a tool which helps inform the surgeon at each step wh le progressively cutting and evulsing the peripheral branches that what has been left behind is still adequate for moving the muscles of all r most of the face—leaving even a weak response to suffice for adequate movement later on

Postoperative period and aftermath

Many of the facial nerve dilution operations as well as the phenol alcohol techniques lead to an initial stage of facial weakness if not actual facial paralysis In all these procedures the facial nerve usually returns to normal within days or weeks[1] and the spasm disappears—only to reappear usually before a year is over

In the operation described above subtotal facial nerve evulsion facial weakness may also set in immediately postoperatively It disappears entirely or to a great extent within weeks or a few months The facial spasm however d sappears immediately When the period arrives at which

[1] In one of our pat ents such facial paralysis las hours while in another it lasted over 2 months cases, the treatment was inject on of p main trunk region (Sade 1973)

the facial spasm usually reappears, i e after about 6–12 months, slight spontaneous movements may occur now and then These symptoms are, however, so slight that they are hardly troublesome to the patient—by no means anything like the original condition Altogether, subtotal evulsion of the facial nerve is therefore an operation which in the long run, may deprive the patient of a small part of his facial integrity and of the greater part of his spasm—a compromise between operations giving a more severe recurrence with no facial weakness, and operations giving no recurrence at all but significant facial weakness

The results of treatment in hemifacial spasm should be evaluated post-operatively after at least 2 years—at that time all that could recur will already have set in I have observed 5 patients (2 men, 3 women) treated by subtotal evulsion of the facial nerve for a period of over 2 years Their average age is 54 years and the average post operative time 31 months Two of them had slight facial weakness post-operatively for some weeks, two had moderate weakness for 2 months and the fifth had significant facial weakness for 5 months In all cases facial movements have returned, though a very slight facial asymmetry remains—which however is mostly observed only by the initiate, and does not disturb the patient The facial spasm disappeared immediately in all 5 patients—though after 9–12 months slight spontaneous movements returned in three These appear rather infrequently and leave these patients undistressed The fifth patient—who had the most severe postoperative facial weakness—has had no recurrence of spontaneous movement As all these patients are now over 2 and close to 3 years postoperative, the chances are that these results are stable

DISCUSSION

The nature of hemifacial spasm

Though fatigue and tension may aggravate the spasm, this entity is not considered to be of psychosomatic origin because of its strict and neurological localisation, lack of response to tranquilizers or psychotherapy and the presence of contractions during sleep (I have even seen the face contract under general anesthesia!)

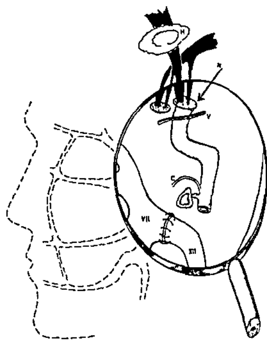


Fig 4 Schematic drawing to show why the origin of spontaneous electric charges in hemifacial spasm is most likely nuclear *VII*, peripheral part of facial nerve *XII* hypoglossus anastomosed to peripheral part of facial nerve, *C*, chorda tympani, *V*, blood vessel near brain stem (Jannetta) *N*, facial nucleus *H* hemorrhage (Ehni & Woltman)

The disappearance of the contraction after facial nerve resection with or without hypoglossus anastomosis precludes the origin of the spasm as occurring at a region peripheral to the stylo mastoid region (Fig 4) Diamant et al.'s (1967) observation of concomitant stapedial contraction as well as hyper-secretion from the submandibular glands with facial spasm places the lesion above the second facial genu How high above the genu do the spontaneous electrical discharges originate? (Fig 4) Do they originate centrally as in epilepsy or more peripherally, as in spontaneous vertiginous attacks in Meniere, or as in painful discharges from trigeminal neuralgia? The occasional beneficial effect of Tegretol or antiparkinsonian drugs—which I have seen in some but not all patients, suggests a nuclear origin of the spontaneous

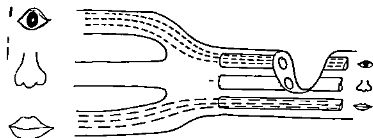


Fig 5 Diagram showing the specific regional representation theory whereby each peripheral branch represents a specific facial region and that such a representation continues in the main facial stem

discharge (Bloom, 1963) At the same time, is evidence which eliminates the cortex as the source of the spasms—thus 2 patients afflicted with facial spasm suffered a stroke involving the face *but not affecting the spasm* (Ehni & Woltman, 1945) There is also experimental work supporting the non involvement (Fowler, 1939, Ehni & Woltman, 1945) of the ... The main evidence as to the trigger zone, ...lusive as it is, indicates the region above second facial genu up to the facial nucleus the pons as that where the spontaneous electrical activity originates Jannetta's (1970) observation of blood vessels compressing the intracranial part of the facial nerve is in agreement with this belief Thought, however, should also given to the theory that the 4-S system—a ...em which controls much of our finer moves—is defective (McCabe, 1970)

Facial nerve anatomy as seen through surgery for hemifacial spasm

The peripheral branches of the facial nerve are believed traditionally to carry impulses to definite and specific regions of the face (May, 1970) The same representation is supposed to be present in the main facial trunk throughout its length, (including also the middle ear) We shall term this concept the *specific regional representation theory* (Fig 5)

The operations performed for facial spasm on the main trunk (Scoville, 1955) of the facial nerve consist essentially in dividing an important part of its width together with the fibres which pass through it (Fig 2) This operation would bring about a total *selective* or *regional* paralysis of those parts enervated by the cut fibres, if the specific regional representation theory reflected the intimate anatomy of the facial nerve trunk However, such selective paralysis does not happen

Furthermore, as mentioned previously, those operations which sacrifice several of the peripheral facial nerve branches would also lead to specific regional paralysis if each branch were to represent a specific region—and this does not happen either The observation that no specific regional facial paralysis occurs, either when the

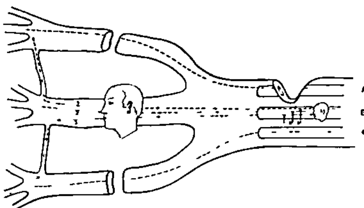


Fig 6 Diagram showing peripheral anastomosis through which pass the result of which representation of the peripheral ...nuation

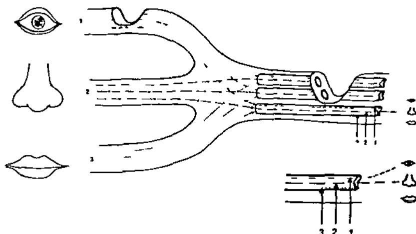


Fig 7 Diagram showing intermingling and intermixing of various peripheral branches in the main stem which bring about wide representation (fibres from 1 & 2 & 3) by even a small nerve bundle in the main stem

main trunk is partly divided or when some of the peripheral branches are divided, should therefore lead us to reject the specific regional representation theory What seems to be the case is that each peripheral branch "represents" a large part of the whole face When some are cut, the remaining one or two suffice to carry movements to the entire face—this can probably come about, as already mentioned, only through a very extensive peripheral anastomosing net work (Fig 6) At the same time the fact that only a small section of the main trunk also suffices to enervate the whole face indicates that each small section of the main trunk also has enough representative axons to represent a major part of the face This could be achieved through an intraneural arrangement of axonal mixing and intermingling, so that the various peripheral regions represented in the main trunk are arranged in several bundles—each of them representing the whole face or its greater part (Fig 7)

These anatomical conclusions derived from the various surgical observations have also received conclusive support from the experimental work of Duel & Fowler (1939) as well as experiments of Harris (1968) and from microanatomical dissections of the facial nerve, by Sunderland & Cossar (1953), and White & Verma (1973)

This subdivided distribution of the various axons into a multiple bundle arrangement—each bundle carrying axons for the whole face—has another interesting aspect A serious doubt arises as to whether such a very complicated and sophisticated micro arrangement can be approximated surgically, after being traumatically severed The "high distribution factor" of each of the fibres in the trunk makes exact matching of single nerve sections highly improbable The traditional specific regional representation concept allows for a more or less approximation of the various intraneural "specific regions" when a traumatic severed main trunk is resutured However, if we consider this specificity to be widely distributed among various bundles and limited more to the individual axons than to a whole group of them, surgical approximation becomes a rather remote practical possibility This consideration should have a bearing on the perfection or imperfection of the results of facial nerve suture or grafting in traumatic cases, and explain much of the associated and mass movements observed after such reconstructive surgery—as perfect as it may seem to be technically

It is also not certain that the amelioration seen after single severed peripheral facial branches are sutured is actually due to this surgical procedure and not to messages arriving through peripheral anastomosis

Treatment of hemifacial spasm

This affliction can be slight or severe and the patient's attitude to his ailment varies also from relative nonchalance and acceptance to complete fixation on the contractions and to the attention

hich the community pays to this phenomenon spontaneous cure is indeed a rare occurrence (Ehni & Woltman, 1945).

After having observed and treated 21 patients, I do not believe that all cases should be treated in the same way The mild cases should be treated —at the beginning at least—with Tegretol, while in more advanced cases in elderly patients phenol injection into the facial trunk should be considered Severe cases appearing in relatively young, active, people should be treated by surgery When proposing surgery, one should explain to the patient the nature of the disease and the factors involved, i e , spasms, paralysis and recurrence and the compromise described above, of—the possibility of barely perceptible and slight facial weakness associated with occasional recurrence of mild spasms—as well as the other treatments and alternatives

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I would like to thank Mr Ben Jalid for his help in making the drawings and Mr Falik for the photography

ZUSAMMENFASSUNG

Die verschiedenen chirurgischen Verfahren, die im Falle eines hemifazialen Spasmus ausgefuhrt werden, wobei ein Teil des fazialen Nervs seziert wird, zeigen an 1 Eine vermischte intrafaziale Anordnung der verschiedenen Nervfilamente Diese Anordnung weicht von der klassi-

funktionell einen grosseren Teil des Gesichtes darstellen kann 2 Die Anwesenheit eines weiteren peripheralen anastomotischen Netzwerks, das die peripheralen Zweige in solch einer Weise verbindet, dass ein Zweig funktionell die anderen Zweige im Falle einer Abtrennung representiert

Auf Grund dieser anatomischen Beobachtungen und den haufigen Rezidiven der fazialen Spasmen nach meist vorgeschriebenen chirurgischen Eingriffen wird in manchen Fällen die Ausrottung der meisten peripheralen Zweige empfohlen Der mandibulare Zweig und ein oder zwei oberer Zweige werden zurückgelassen Dies wird als chirurgische Kompromissverfahren betrachtet, das eine leichte Fazialschwachung sowie auch mögliche, milde und verhaltnismässig seltene spasmodische Bewegungen ergibt Weitere theoretische Folgerungen aus der Anordnung der fazialen Filamente bedeutet ‚a priori' eine Begrenzung der chirurgischen Ergebnisse der fazialen Nervverbindung und Verpflanzung in traumatischen Fallen

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CAPILLARY AND SHUNT BLOOD FLOW IN THE NASAL MUCOSA OF THE CAT

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Abstract Radioactive microspheres (15±5 μm) were used to investigate capillary and shunt blood flow in the nasal mucosa of the cat Capillary flow was about 0 5 ml/g min An abundant arterio venous shunting was simultaneously recorded Shunt flow was 57+11% of total blood flow Sympathetic stimulation resulted in a greater reduction in shunt flow than in capillary flow, indicating that increases in sympathetic nerve activity will result in a redistribution of flow from shunt to exchange vessels

Morphological studies of the nasal vascular bed in man and cat have demonstrated the presence of arterio-venous anastomoses (AVA) in the l mucosa (Dawes & Prichard, 1953, Rossatti, 1954, Cauna, 1970) The AVA were frequently encountered in the deeper sections of the nasal respiratory mucosa and usually near the nasal glands (Fig 1A)

Previous experimental studies on the microcirculation in the nasal mucosa have provided information regarding the nervous control of resistance, capacitance and exchange vessels (cf Änggård, 1974, Malm, 1973) However, no information is available on the quantitative importance or the nervous control of arterio-venous shunt flow in the nasal vascular bed

Injection of radionuclide-labelled microspheres into the circulation of animals provides a possibility of measuring the proportion of blood flow to any organ or the regional distribution of the blood flow within an organ (cf Rudolph & Heymann, 1972, Wagner et al, 1969) An important assumption for the technique is that all microspheres are trapped in the small vessels If AVA are present these will allow an escape of the spheres, which will then appear in the venous effluent in proportion to shunt flow

In the present investigation this method was used to demonstrate the presence and quantitative importance of shunt flow in the nasal vascular bed of the cat

MATERIALS AND METHODS

Experiments were conducted on 17 cats (2.3-4 5 kg) anesthetized with chloralose-urethane (50 mg/kg+100 mg/kg i v) A diagrammatic presentation of the experimental arrangement is shown in Fig 1B The trachea was cannulated. The pressure in the left femoral artery was measured by a Statham pressure transducer (P23A) and recorded on a Rikadenki multichannel recorder Rectal temperature was kept constant at 38°C by heating lamps The right cervical sympathetic nerve was dissected free from the vagal nerve and transected Sympathetic stimulation was performed in the cranial direction using a bipolar silver electrode The stimuli were monophasic square wave pulses (6V, 1 msec) delivered by a Grass model S4 stimulator Stimulation frequencies of 0 5 and 1 0 imp/sec were used For the collection of blood from the nose the right sphenopalatine vein, which drains the

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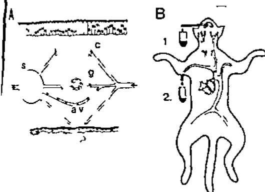


Fig 1 Schematic drawing of the experimental set up and the vascular arrangement in the nasal mucosa

posterior part of the nasal cavity (Dawes & Prichard, 1953), was exposed by a transorbital approach To gain access, the eyelids were removed and the eyeball opened and emptied The sphenopalatine vein was isolated from communicating veins and cannulated retrogradely from the deep facial vein in the angle of the mouth, using a polyethylene tube (PE 160) The tip of the cannula was positioned at the level of the nose Reference arterial blood samples were collected from the right subclavian artery, which was cannulated with a polyethylene tube (PE 160) During the measurements the blood flow from the artery was adjusted by a clamp to a flow similar to that measured from the sphenopalatine vein Heparin (Vitrum) 1000 IU/kg was given i v every second hour Blood was re infused into the left cephalic vein

For the injection of the microspheres into the blood stream a catheter (PE 90) supplied with a three way stopcock was introduced into the brachiocephalic trunk (4 cats) or positioned in the ascending aorta (4 cats) via the right femoral artery The positions of the catheters were confirmed by dissection at the end of the experiment As it was found difficult to determine the position of the catheter within the aortic arch or brachiocephalic trunk with certainty, the left atrium was cannulated in later experiments In 9 cats artificial respiration was given (B Braun Melsungen respirator) and a left sided thoracotomy was performed A polyethylene catheter (PE 20) was introduced into the left atrium through a small pulmonary vein Arterial blood samples were taken repeatedly and analysed for pH, pCO_2 and pO_2 (BMS 3 Mk 2, PHM 71, Mk 2, Radiometer, Copenhagen) These parameters were kept normal during the experiments by means of artificial respiration

Microspheres with a diameter of 15 ± 5 µm, labelled with ^{141}Ce or ^{51}Cr, suspended in 10 % dextran were used (3M Company, St Paul, Minnesota) The microsphere stock solutions were resuspended using a mixer immediately prior to aspirating 0 15–0 5 ml into a 1 ml syringe The syringe was rapidly attached to the three way stopcock on the arterial catheter and the injection was performed immediately The injection syringe and catheter were flushed with 0 5 ml of 10 % dextran in a second syringe previously attached to the stopcock At the start of the injection venous blood from the nose and arterial reference samples were collected in 3 ml vials during 1½–2 min Blood flow was measured as drops/min and the collected samples were later weighed After 5–10 min the procedure was repeated with a differently labelled bolus of microspheres In each cat either ^{51}Cr or ^{141}Ce labelled microspheres were injected once in order to investigate the regional distribution of capillary blood flow within the nasal mucosa, while the other isotope was injected 3–4 times when only arterio-venous shunting was measured

After each experiment the maxilloturbinal (inferior concha) and ethmoturbinal (middle concha) on each side was removed and weighed The separate blood and tissue samples were counted for 10 min in a gamma ray spectrometer (Packard Tricarb gamma spectrometer 3320)

The radioactivity in the arterial ($A^{\text{counts ml}}$ and venous ($V^{\text{counts ml min}}$) blood samp measured The percentage shuntin

ing) and shunt flow were then calculated according to the following equations

$$\% \text{ shunting} = \frac{100 \times V^{\text{counts ml min}}}{A^{\text{counts ml min}}} \quad (1)$$

shunt flow = Total flow × % shunting (2)

Capillary flow (C) was then determined as the difference

Capillary flow = Total flow minus shunt flow

The radioactivity (counts/g) in the tissue samples ($T^{\text{counts g}}$) was also measured and regional capillary flow (C_t) calculated as follows

$$C_t = \frac{T^{\text{counts g}}}{A^{\text{counts ml min}}}$$

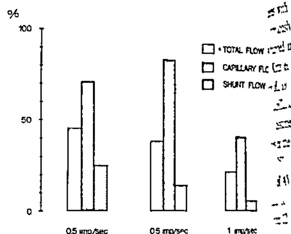


Fig 2 The reduction from resting values in total, capillar and shunt blood flow in nasal tissues following sympa thetic stimulation at 0 5 and 1 0 imp/sec Resting flow = 100 %. (Cat 4 1 kg)

RESULTS

A large proportion of the microspheres passed through the nasal vascular bed in all experiments, indicating the presence of shunt flow When the spheres were injected into the aortic arch or the brachiocephalic trunk the number of spheres passing through the microcirculation ried considerably between the different animals ($\bar{X}=44 \pm 24$ %, $n=15$) As it was found t to determine the position of the injecon catheter within the aortic arch or brachiocephalic trunk with certainty, the left atrium was cannulated in later experiments This resulted in smaller variations in results between and within the different animals and a higher proportion of spheres passed through the micro circulation, when compared with results from the previous injection method ($\bar{X}=57 \pm 11$ %, $n=20$, $p<0.05$) When the number of microspheres in each injection bolus did not exceed 1 5 million injections could be repeated 3–4 times without noticeable reductions in blood flow from the sphenopalatine vein

In 5 cats sympathetic stimulation regularly resulted in a decrease in venous outflow, accompanied by a reduction in shunt and capillary flow (Fig 2) At a frequency of 0 5 imp/sec the reduction in shunt flow from resting values ($\bar{X}_s=77 \pm 9$ %, $n=5$) was higher than the reduction in capillary flow ($\bar{X}_c=28 \pm 14$ %, $n=5$, $p<0.001$) or total flow ($\bar{X}_V=56 \pm 9$ %, $n=5$ $p<0.02$)

In 6 cats regional capillary flow within the maxilloturbinal and ethmoturbinal on each side was established To obtain sufficient countin accuracy, when counting the radioactivity from the trapped microspheres in the small amount of tissue of the maxilloturbinal, the number of microspheres in each injection had to be increased (about 2 5–4 million microspheres were required) It should be observed, however, that in addition to nasal mucosa the excised loturbinal and ethmoturbinal also conta sal cartilage and bone tissue The lary flow, expressed in ml/g/min, siderably in the different maxilloturbinal ($\bar{X}=0.61 \pm 0$ turbinal ($\bar{X}=0.51 \pm 0.43$), ($\bar{X}=0.41 \pm 0.36$, $n=8$), eth 0 41, $n=8$) No significant lary flow was found bet binal and the ethmoturb when both sides were c

DISCUS

In the present study were recorded by simu nous outflow and the 15±5 μm spheres t bed in cats

Microspheres with a diameter greater than 10 μm have previously been shown to be almost completely trapped in the microcirculation of the kidney (Katz et al, 1971) and the heart (Buckberg et al, 1971), indicating that they do not pass the capillaries within these organs It is reasonable to assume that they also fail to pass the capillaries in the nasal vascular bed Dawes & Prichard (1953) demonstrated and measured the diameter of AVA in neoprene casts of the nasal vascular bed of the cat They observed diameters from 12–60 μm, which indicates that the microspheres used in the present experiment are suitable for the study of AVA and capillary blood flow in the nasal vascular bed

The present, results which show that about 60 % of the spheres passed through the nasal vascular bed, might seem very high Methodological errors affecting the microsphere injection technique have been analysed by Buckberg et al (1971) and their suggestion for reducing these errors have been followed in the present study Thus the number of microspheres in each blood sample greatly exceeded 1000, making random variations in the distribution of the microspheres of minor importance Thus the difference in results obtained following injections into the aortic arch or brachiocephalic trunk and the left atrium, respectively, is probably due to uncomplete mixing of the spheres at the junction of the subclavian and common carotid arteries following the first type of injection This implies that the injection should preferably be performed into the left atrium The present results on shunt flow thus represent the functional consequences of the rich abundance of AVA in the nasal mucosa as described in morphological studies (Dawes & Prichard 1953 Rossatti 1954 Cauna 1970)

Labelled microspheres have previously been used to estimate blood flow in otorhinologic tissues including the nasal mucosa (Abe & Jackson, 1972) However the present finding that more than 50 % of the spheres appeared to pass through AVA invalidate any calculations of total nasal blood flow with this technique and implies that the regional distribution of spheres in the nasal mucosa will solely reflect the capillary flow

When capillary flow was investigated in this way no clearcut difference between the two nasal cavities, the ethmoturbinal or maxilloturbinal area could be found This indicates that the microcirculation was unaffected by the operation procedures on the right side Capillary flow averaged 0 5 ml/g/min, which is in agreement with previous results in the dog (Clairmont et al, 1973)

Morphological studies of the nasal mucosa have indicated an adrenergic, as well as a cholinergic, innervation of the arterial section of the AVA (Cauna, 1970) The difference in the relative response between AVA and capillary flow to sympathetic stimulation found in the present experiments indicates that AVA in the nasal microcirculation reacts in a similar way to the AVA in the skin and paw of the dog, where AVA responded more to α receptor stimulation and blockade than did the capillary section (Spence et al, 1972)

Thus the present study provides evidence of an unusually abundant shunt flow in the nasal vascular bed and indicates that increases in sympathetic nerve discharge will result in a redistribution of flow from shunt vessels to exchange vessels

ZUSAMMENFASSUNG

Unter Verwendung von radioaktiven Mikrospharen (15 ± 5 μm) wurde die Kapillar und Shuntblutzufuhr an der Nasenschleimhaut der Katze bestimmt Die Kapillarblutzufuhr betrug etwa 0 5 ml g min Gleichzeitig wurde eine reichliche arterio-venose Shuntblutzufuhr registriert

Die Shuntblutzufuhr betrug 57 ± 11 % der gesamten Blutzufuhr Sympathische Stimulation fuhrte zu einer starkeren Verminderung der Shuntzufuhr als der Kapillarzufuhr Diese Befunde deuten darauf hin dass eine Zunahme der sympatischen Nervenaktivität eine Neuverteilung der Blutzufuhr von den Shuntgefassen zu den Austauschgefassen zur Folge hat

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TOMOGRAPHIC EXAMINATION OF THE VERTICAL PART OF THE FACIAL CANAL IN CASES OF BELL'S PALSY

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Abstract In 41 patients with Bell s palsy of different degrees of severity a tomographic examination has been made of the vertical segment of the aqueduct of Fallopius from the second elbow to the stylomastoid foramen The width and length of this segment of the canal and the occurrence of mastoid cellulae around the canal displayed large variations There were, however, no significant differences between the affected and the sound side in the individual subject A comparison between the findings in the palsy group and those in 30 healthy controls disclosed no significant differences in canal anatomy The results of the study suggests that variations in the anatomy of the vertical segment of the canal are of only minor importance, if any, for the occurrence of Bell s palsy or for the extent of the damage to the nerve

The cause of Bell s palsy is unknown According to the most quoted of the theories on the background of the disorder the precipitating factor is ischaemia followed by impairment of the circulation within the facial nerve (Hilger, 1949, Kettel, 1959, Jain & Sharma, 1964, McGovern et al, 1972) It is considered that ischaemia in the nerve can arise through exposure to cold or inflammation (Collier, 1951 Kettel, 1959, 1963, McGovern et al, 1963), but also as a result of intravascular interruption of the nerve s venous supply (Fowler Jr, 1963) However, in facial palsy of the zoster-type, Devriese (1968) found evidence that oedema and pressure were of subordinate importance

Because of oedema, which is caused by, or which causes, ischaemia, the course of the nerve through a narrow rigid canal in the bone would worsen the paralysis, and also the prognosis

Primary oedema is regarded as a cause of, and an aggravating factor in, ischaemia by Langworth & Taverner (1963), while Blatt & Freeman (1966) found oedema and inflammation in Bell s palsy unaccompanied by ischaemia According to Hilger (1949), Sullivan & Smith (1950) and Kettel (1959) primary ischaemia, which they found usually to be located in the vicinity of the stylomastoid foramen, results in venous stasis and oedema, this is followed by secondary ischaemia, the collaterals in the bone canal then being compressed, thereby causing further impairment of the circulation There is no objective proof that the canal lumen is a factor of importance in the development of the paralysis

In conformity with the above hypothesis *decompression of the canal to relieve* pressure on the facial nerve is performed as a treatment for peripheral idiopathic facial palsy (Ballance & Duel, 1932, Sullivan & Smith, 1950, Jongkees, 1972, Pulec, 1972*a*) The value of, and particularly the indications for, this form of therapy have, however, been questioned by several researchers (Blatt, 1965, Miller, 1967, May & Hawkins, 1972, Diamant et al, 1972, Haglund, 1972), as conservative treatment has led to recovery of nerve function in 80–91 % of the cases (Hilger, 1949, Laumans & Jongk 1963, Langworth & Taverner, 1963, Ha 1972)

According to neurophysiological

usual site of the paresis in Bell's palsy is the segment of the nerve from the geniculate ganglion to the stylomastoid foramen (Jepsen, 1965, Zilstorff-Pedersen, 1965, Jongkees, 1965, May, 1970) Even in subjects with normal function of the facial nerve individual deviations in the course of the nerve and considerable variations in the diameter of the canal lumen and the length of the segment have been observed in this segment of the canal (Kettel, 1959, 1963, Fowler, 1961, Miehlke, 1965, Ericson & Liliequist, 1973) It is not known whether the size of the canal lumen in persons with Bell's palsy lies outside the normal range or whether the anatomy of the canal differs in subjects with persistent paralysis from that in persons who recover completely and rapidly, nor is it known whether there are such anatomic differences between the paralysed and the sound side in the same individual With the object of throwing some light on these obscure points we have carried out a study of the course of the vertical segment of the facial canal in subjects with Bell's palsy of different degrees of severity

CASE SERIES

The study was performed on 41 subjects—16 men and 25 women—aged from 15 to 81 years admitted to the Department of Otorhinolaryngology, University Hospital, Umeå, with a diagnosis of Bell's palsy (peripheral idiopathic facial palsy)

The patients were submitted to (1) a clinical examination, (2) tests of chorda tympani function with determinations of taste and salivary secretion, (3) neurophysiological tests including EMG, and latency and threshold determinations

(1) *Clinical examination*

Symptoms were registered and an evaluation of the paralysis in the three main branches of the facial nerve was made

(2) *Chorda tympani function*

(a) *Salivary gland function* The function of the secretory fibres of the chorda tympani was tested by a method devised by Wiberg (1971)

(b) *Taste function* The function of the afferent fibres of the chorda tympani was examined by electrogustometry by Kraarup's method and by semiquantitative threshold determinations by a modification of Bornstein's method (Wiberg, 1971)

(3) *Neurophysiological tests*

Electromyography (EMG) was performed to examine the motor innervation of the mimic muscles, latency and threshold measurements were performed by routine methods at the Neurophysiological Laboratory

The results of the above examinations were used in a four-grade classification of the series according to Diamant et al (1972)

Group I Complete recovery in 3 months

Group II Complete recovery in 3–12 months

Group III Incomplete recovery after 12 months, normal facial appearance at rest but defective function in spontaneous movements

Group IV Incomplete recovery after 12 months, paralysis obvious at rest and/or marked synkinesis, and/or marked loss of motor units as seen in EMG 12 months after onset of paralysis

The sex distribution for group I+II and group III+IV is shown in Table I

The palsy series was compared with a control group consisting of Umeå University students of both sexes with no history of paralysis or chronic ear disease A detailed description of the control group has been given in an earlier paper (Ericson & Liliequist, 1973)

Table I *Sex distribution of 41 patients with Bell's palsy divided according to severity of the nerve involvement*

	Men	Women	Total
Slight–moderate (Group I+II)	12	17	29
Severe total (Group III+IV)	4	8	12
Total	16	25	41

Tomographic examination

The vertical segment of the facial canal from the outer bend on the second elbow to the stylomastoid foramen was examined by tomography in lateral projection by the method described by Ericson & Liliequist (1973) After deciding the depth of the section in preliminary tomograms the definitive examination was planned This consisted of 5 tomographic sections parallel to the sagittal plane at intervals of 1 mm An example of a tomographic cut through the vertical segment of the canal is given in Fig 1*a* There was an enlargement factor of 1 3 due to the ray geometry The films were taken with a Polytome tomograph, and the hypocycloid motion of the apparatus was used

The tomogram on which the vertical segment of the facial canal was most distinctly visualized and on which the calibre of the canal was also widest was used as a basis for the measurements The contour of the canal was marked with a sharp pencil, and the lumen was measured at the most proximal level (A) midway along the segment (B), at a point (C) midway between B and the stylomastoid foramen (D) The length of the segment A–D was also measured The distances recorded are shown in Fig 1*b*

All the measurements were performed by one observer with a magnifying glass bearing a scale graduated in tenths of a millimetre

The occurrence of mastoid cellules along the canal lumen was also graded according to the scale none (0), moderate number (1) and numerous (2)

Table II *Means and standard deviation for the dimensions of the vertical segment of the aqueduct of Fallopius measured on lateral tomograms in 41 patients with Bell's palsy, millimetres*

	Affected side		Sound side		Level of significance
	Mean	S D	Mean	S D	
Width of lumen at					
A	1 76	0 49	1 80	0 41	Not significant
B	2 10	0 44	2 15	0 48	Not significant
C	2 34	0 53	2 40	0 50	Not significant
D	3 17	0 92	3 18	0 75	Not significant
Minimum width	1 73	0 49	1 80	0 40	Not significant
Length of segment	16 07	2.31	15 85	2 08	Not significant

Analysis of the tomographic method

Analyses of the tomographic visualization of anatomic details in the temporal bone and of the errors ascribable to the identification of reference points and the measurements of the distances with the technique used have been reported elsewhere (Eckerdal, 1971, 1973, Ericson & Liliequist, 1973) The error of the method was small and negligible for this comparative study

Statistical analysis

The calculations were performed on computors, and the usual statistical principles were used Tests of parametric variables were carried out by Student's *t* test and of nonparametric variables by the χ^2 analysis

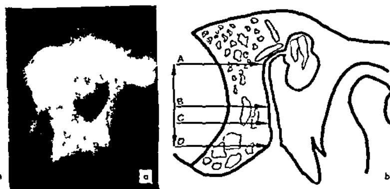


Fig 1 (*a*) Tomographic visualization of the vertical segment of the aqueduct of Fallopius from the second elbow to the s mastoid foramen Palsy (*b*) The vertical se canal showing points A B C distance A–

Table III *Mean difference (mm), standard deviation of the mean difference and coefficients of correlation between the affected and the sound side in the same subject for dimensions of the vertical segment of the aqueduct of Fallopius*

Width of lumen at point												Length of segment		
A			B			C			D					
d	SD	*r*	*d*	SD	*r*	*d*	SD	*r*	*d*	SD	*r*	*d*	SD	*r*
−0 01	0 25	0 66***	0 01	0 40	0 40**	−0 07	0 55	0 19	−0 01	0 67	0 61***	0 27	1 43	0 79 **

RESULTS

The results are reported in Tables II–VII and Figs 1–4

The length of the vertical segment (A–D) of the facial canal, the size of the canal lumen in the sagittal direction for the levels, A, B, C and D and for the narrowest part of the lumen found empirically in the section, for both the paralysed and sound side, are given in Table II The means and standard deviation for the variables were numerically equal on the two sides As is seen from the standard deviation and Figs 1–4, however, there were large variations the canal lumen and in the length and also the orientation of the canal in the sagittal plane

To examine more closely whether there was any intra-subject difference between the paralysed and the sound side, the mean difference and the coefficient of correlation between the two sides were computed (Table III) The mean differences in the calibre of the canal between the paralysed and the sound side were small at the four levels of measurements For the length of the vertical segment the difference was slightly larger but not statistically significant As the standard deviations and correlation coefficients show, the differences between the sides in the individual subject could be quite large

To examine whether variations in the severity of the paralysis might be due to differences in canal size the mean diameter of the canal lumen at different levels and the mean length of the canal segment were calculated for subjects in group I and in groups III–IV, as was the mean intra subject difference between the two sides for groups I and III–IV There was no statistically significant difference between subjects of these two groups as regards the canal parameters examined (Tables IV and V)

A comparison was made of the lumen size and the length of the vertical segment in the palsy and control groups (Table VI) The only significant difference was in respect of the lumen diameter at the stylomastoid foramen, which was significantly wider in the controls this difference was found only in the men (Table VI)

Because of the large individual variations it was examined in analogy with Table IV, whether there was any difference in the canal anatomy between, on the one hand, the controls and on the other, mild and severe grades of palsy

Table IV *Means and standard deviations for the width and length of the vertical segment of the aqueduct of Fallopius in 17 patients with Bell's palsy group I and 12 patients in groups III+IV millimetres*

	Group I		Groups III+IV		Level of significance
	Mean	SD	Mean	SD	
Width of lumen at					
A	1 84	0 36	1 87	0 34	Not significant
B	2 28	0 56	2 27	0 44	Not significant
C	2 50	0 60	2 53	0 50	Not significant
D	3 28	0 65	3 07	0 77	Not significant
Length of segment	16 00	1 60	15 93	2 57	Not significant

Table V *Mean difference between affected and sound side in 17 and 12 patients with unilateral peripheral Bell's palsy group I and III+IV*

	Width of lumen at								Length of segment	
	A		B		C		D			
	d	SD	d	SD	d	SD	d	SD	d	SD
Group I	−0 02	0 28	0 23	0 83	−0 12	0 60	−0 08	0 55	0 22	0 86
Groups III+IV	−0 01	0 40	−0 19	0 92	0 07	0 35	0 09	0 73	0 61	1 69

There were no significant differences between these groups except as regards the canal orifice (Table VII) In this comparison too, the difference was limited to the male subjects

Comparison of the occurrence of mastoid cellules around the vertical segment of the facial canal within the palsy group and between this and the control group disclosed no difference in either case

DISCUSSION

According to the tomographic examination of the vertical segment of the aqueduct of Fallopius the size of the lumen and the length and orientation of the canal in the sagittal plane varied fairly widely in the patients with Bell's palsy of different degrees of severity, on both the affected and the sound sides This is particularly

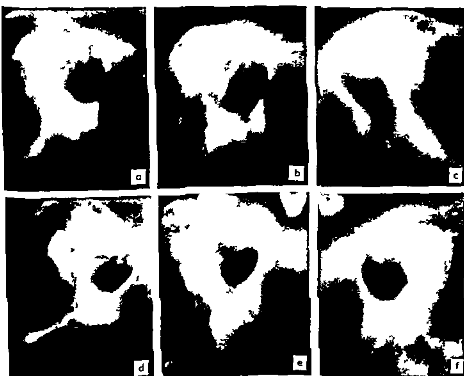


Fig 2 Tomograms of the vertical segment of the aqueduct of Fallopius in 6 patients with un (a–c) Palsy group I (d–f) Palsy grou

Table VI *Comparison between 41 patients with peripheral Bell s palsy and 30 healthy controls with ... the aqueduct of Fallopius*

	Both sexes					Men				
	Palsy		Controls		Level of significance	Palsy		Controls		Level of significance
	$\bar{x}$	S D	$\bar{x}$	S D		$\bar{x}$	S D	$\bar{x}$	S D	
Lumen width at A	1 76	0 48	1 93	0 53	Not sign	*1 83*	0 50	2 06	0 42	Not sign
B	2 10	0 43	2 07	0 37	Not sign	2 06	0 40	2 18	0 38	Not sign
C	2 34	0 52	2 36	0 61	Not sign	2 33	0 58	2 59	0 49	Not sign
D	*3 17*	*0 91*	3 89	1 29	** (t = −2 73)	3 22	0 92	4 24	1 26	** (t = −2.73)
Length of segment	16 07	2 28	16 60	3 25	Not sign	16 28	2 84	17 44	2 24	Not sign.

true of the canal length and the aperture in the stylomastoid foramen In practically all subjects, however, the lumen was narrowest at the top and increased steadily in width in the peripheral direction The results are consistent with those of a corresponding study of healthy subjects (Ericson & Liliequist, 1973) Chouard et al (1971) state that the diameter of the mastoid part of the facial canal is largely constant, but that there is an important narrowing where the nerve leaves the canal at the stylomastoid foramen This view was also advanced by Cawthorne (1946) and Sullivan & Smith (1950), but is not supported by the results of the present study or that of Lindeman (1960) It is possible that these authors misinterpreted the funnel shaped orifice in some subjects as a narrowing (Figs 2*a* & *b*, *c* and 3*b*, *d*, *e*) So far as we are aware there

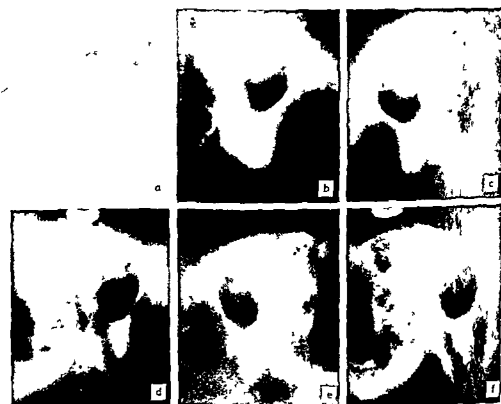


Fig 3 Tomograms of the vertical segment of the aqueduct of Fallopius in 6 patients with unilateral Bell s palsy (*a–c*) Palsy group III (*d–f*) Palsy group IV

respect to dimensions of the vertical segment of

Women				
Palsy		Controls		Level of significance
x	S D	x	S D	
1 70	0 46	1 70	0 40	Not sign
2 13	0 45	1 90	0 29	Not sign
2 35	0 48	2.0	0 60	Not sign
3 13	0 90	3 36	1 10	Not sign
15 91	1 69	15 50	4 24	Not sign

is no previous study such as the present one or that performed on healthy subjects (Ericson & Liliequist, 1973)

With one exception no significant difference in the canal anatomy was observed in comparisons between the affected and sound sides, or between the affected side and the control group The exceptional case concerns the variable for the stylomastoid foramen, which was significantly narrower in men with Bell s palsy than in the controls Because this difference was bilateral and limited to this variable and one combination, the observation must be regarded as unreliable

The tomographic examination of the vertical segment of the canal points fairly strongly to the fact that the variation in the width of the lumen and in the length and proximity of the canal segment to the mastoid cellules in this segment itself has no bearing on the occurrence of Bell s palsy, or on the severity of the nerve impairment Whether the fact that the nerve passes through a rigid canal in the bone, and this alone, tends to cause palsy cannot, however, be ruled out on the basis of this study, since there may be co-variation between an aetiological factor and properties of the canal other than its anatomical shape Nor can it be excluded that variations in other segments of the canal than the ones examined are of aetiological significance

Because of the small number of subjects with severe palsy and because the quantitative grading of the paralysis was based on several variables, caution should be observed in evaluating the importance of variations in the anatomy of the canal as an aggravating factor

We have observed that the horizontal part of the canal is narrower than the vertical segment, which gradually widens towards the stylomastoid foramen (cf Fig 4) That the width of the canal is not of primary aetiological significance is suggested also by the fact that oedema in the facial nerve in Bell's palsy is most extensive in the vertical segment (Pulec, 1972*b*), where we have found that the canal is widest The observations that the nerve in the vertical segment takes up only about 35–50 % of the canal lumen (James, 1961) and that the canal is narrower in the horizontal segment, irrespective of the fact that the lesions usually occur in the vertical part of the nerve (Jepsen,

Table VII *Difference in the mean width of lumen and length of the vertical segment in 30 healthy controls and 29 patients with Bell's palsy millimetres*

	Difference in means				
Point	A	B	C	D	Length
Control Group I	0 14[a]	0 03[a]	0 04[a]	0 64[a]	0 60[a]
Control-Groups III–IV	0 20[a]	0 14[a]	0 09[a]	0 89[a]	0 33

[a] None of the differences was significant



Fig 4 Tomograms of the vertical (*a*) and segments of the aqueduct of Fallopius horizontal (←) segment is narrower vertical segment

1965, Zilstorff-Pedersen, 1965, Jongkees, 1965, Miller, 1967, May, 1970), also suggest that the peculiarities in the canal's anatomy are not of critical importance for the occurrence of Bell s palsy and its prognosis Conflicting with this view is the observation by Fisch & Esslen (1972) on exposing the canal that in 11 out of 12 cases of Bell's palsy the maximum nerve oedema was located between the geniculate ganglion and the porus acusticus internus

Collier (1951) and Sunderland & Cossar (1953) found that the nerve occupied only 25–50 % of the cross sectional area of the canal, and Lindeman (1960) gave a range of 10 to 50 %, Hilger (1949) and James (1961) found that the nerve has a thick perineural capsule between the stylomastoid foramen and the stapedius nerve This latter observation may be consistent with our experience that the canal lumen widens towards the orifice The amount of connective tissue, the tension in the connective tissue around the nerve and the thickness of the nerve capsule in different sections of the canal might be of significance for the occurrence of palsy, and probably more important than anatomic pecu rities of the bony canal Observations con- ent with this have been reported by, among hers, Hazama et al (1972)

ZUSAMMENFASSUNG

Bei 41 Patienten mit idiopatischer Fazialislahmung (Bell s pares) wurde der Fallopische Kanal vom zweiten Knie bis zum Foramen stylomastoideum mittels Tomographie untersucht Die Untersuchung wies grosse Variationen in Weite und Lange dieses Kanalsegments und auch in der Menge der den Kanal umgebenden Mastoidzellen auf Bei keinem Patienten existierte ein signifikanter Unterschied zwischen der paretischen und der normalen Seite Auch zwischen den Patienten mit Lahmungen und 30 gesunden Kontrollpersonen gab es keine signifikante Unterschiede in der Kanal Anatomie Die Resultate von diesen Untersuchungen deuten an dass Variationen in der Anatomie des vertikalen Kanalteils keine sichere Beziehung zum Verlauf und zur Prognose der idiopa tischen Fazialislahmung haben

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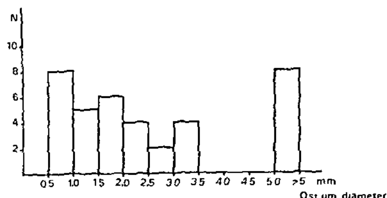


Fig 2 Histogram over the variations of the functional size of the maxillary ostium measured in 37 normal cases

6–7 mm and variations in length have a considerably smaller influence on the gas exchange through the ostium than variations of the diameter or cross sectional area Furthermore, variations in the length cannot be determined in humans with the described method

A possible complication might arise because of air embolism However, the method was only used with subjects in whom the recordings have shown that the cannula was not lying in the mucosa and that the ostium was patent The air insufflation lasted only a few seconds at most

y using different rates of airflow, beginning with the lowest, the pressure rise within the sinus did not exceed 3 cmH_2O (2 20 mmHg) The risk of embolism was completely avoided by taking these precautions

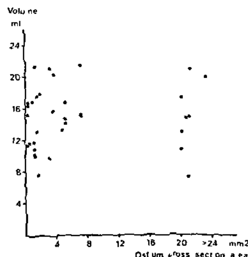


Fig 3 Relation between the volume of the maxillary sinus and the functional size of the maxillary ostium No correlation was found

The method cannot be used for sinuses with ostia larger than 5 mm in diameter, since the pressure increase even with 6 l/min airflow will be too small to be accurately recorded with our equipment However, these cases are relatively few and it is usually sufficient to know that the ostium is larger than 5 mm in diameter since this size will facilitate an unrestricted gas exchange The borderline value for relative insufficiency in the antral ventilation, indicated by a lower oxygen tension in the sinus, is at an ostial dia meter of 2 5 mm (Aust & Drettner, 1974) It is thus of interest to note that the mean ostial size in this investigation was 2 4 mm showing that the borderline for relative ostial insufficiency and the mean ostial diameter are almost identi cal

ZUSAMMENFASSUNG

[illegible] Grösse des [illegible]

[illegible] Luftstrom durch die Kieferhohle geschickt wird. Zu diesem Zweck wird der Luftstrom durch eine Kanüle im unteren Nasengang in die Kieferhohle geleitet und geht durch das Ostium hinaus Die Druckerhöhung ist vom Luftstrom der in den Sinus geleitet wird und von der Grosse des Ostiums abhängig Modellversuche wur[illegible] Ergebnissen wurde ein [illegible]

[illegible] durchschnittliche funktionelle Ostiumsgrösse [illegible] was einem Durchmesser von 2 4 mm entspricht. Es gab keinen Unterschied zwischen Männern und Frauen in der Grosse des Ostiums und auch keine Korrelation zwischen dem Volumen der Kieferhohle und der Grösse des Ostiums

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SCANNING ELECTRON MICROSCOPIC STUDIES OF NASAL POLYPS

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Abstract Nasal polyps yielded specimens, which technically were most suitable for study in the scanning electron microscope A small number of glands and cytoplasm protuberances from the epithelial cells were found 30 % of the total surface was covered by cilia The cilia looked normal with no signs of destruction In half of the specimens, areas with widened intercellular spaces were observed The surface of nasal polyps did not differ essentially from that of the inferior turbinate in patients with perennial rhinitis It was, however, characteristic of the polyps that cells from a transitional like epithelium protruded, dome like at the surface, resembling cobble-stones Besides this a great riation of the surface area of the cells was found, th the occurrence of large cells with a surface area of p to 150 μm^2 Another characteristic feature was the occurrence of an irregular surface with an appearance resembling a hilly country, in which every uneveness consisted of several cells

Nasal polyps are morphologically well defined structures, which represent the most extreme degree of oedema in the mucous membrane The formation of nasal polyps may be due to allergy, but may also have other causes such as mucoviscidosis (Rulon et al, 1963) Extensive histological studies have been made (Andersen, 1943) and also two transmission electron microscopic studies of nasal polyps (Cauna et al, 1972, Jahnke, 1972), but as far as is known to the present authors the problem has not been systematically investigated with the scanning electron microscope (SEM) The ultrastructure of the surface of nasal polyps differs in certain regards from what we previously have described for the inferior turbinate in normals and in patients with perennial rhinitis (Mygind & Bretlau, 1973 and 1974) We therefore find justified to publish our SEM studies of nas polyps

MATERIAL AND METHODS

In 18 consecutive patients removal of a rel tively free-hanging polyp was performed witho local anaesthesia The patients were allerg logically examined, and allergy was demonstrat in 4 cases None of the patients were treat with corticosteroids, locally or systematicall 13 specimens were examined by SEM and were embedded for microscopy in anoptral co trast

The polyps were immediately fixed in a cc 5 % solution of glutaraldehyde, buffered with 0 03 M sodiumcacodylate (pH 7 4) The c molarity of the solution was measured to 5 mOsm/l After 24 hous of fixation the poly were stored in a 0 15 M sodium cacodylate buf (295 mOsm/l) After dehydration in ethyl cohol the specimens for light microscopy we embedded in Epon Sections were cut in Reichert ultra-microtome UM 2 with a Dupc diamond knife Semithin sections were analys in anoptral contrast The specimens for SE were, after dehydration in ethyl alcohol tra ferred to benzene and freeze-dried by the me od described in detail by Nørrevang & Wir strand (1970) The specimens were studied wi a Cambridge Stereoscan S2

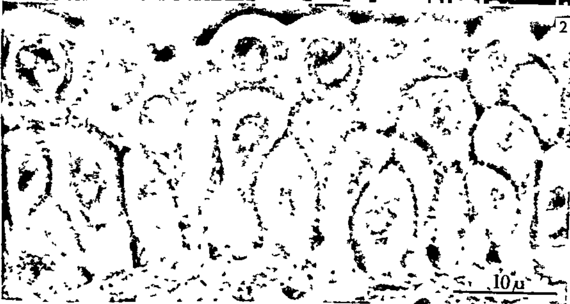


Fig 1 Typical surface of nasal polyp The single cells which resemble cobble-stones are covered by short microvilli The arrows indicate cells penetrating the epithelium probably eosinophil leucocytes × 3 270

Fig 2 Corresponds to Fig 1 A low epithelium of the transitional type with box formed surface-cells It can just be seen that these cells have microvilli on the surface Original magnification × 630

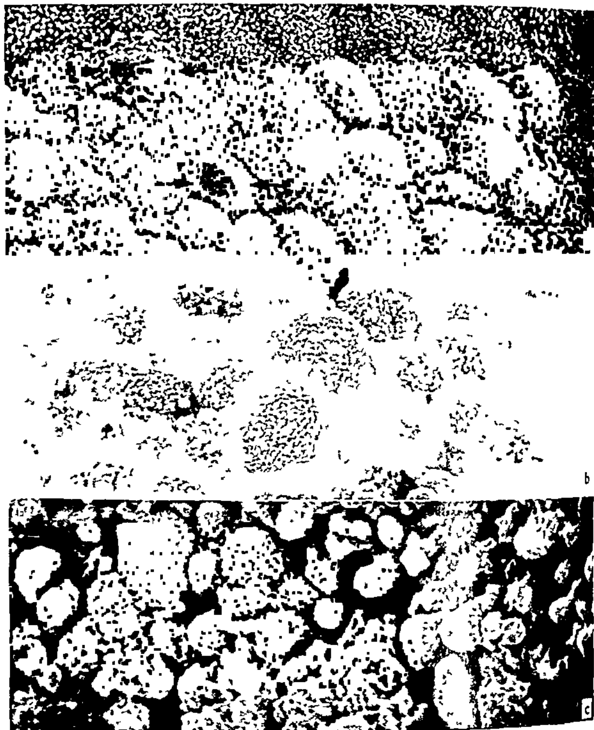


Fig 3a–c Various surface reliefs which can be found on nasal polyps as well as on the inferior turbinate (*a*) The surface is even and covered by microvilli Filled goblet cells can be identified by their nearly smooth surface (*arrows*) They may be surrounded by a ring of microvilli (*b*) The cells are more prominent and the surface irregular, but the terminal bars appear intact The surface of some cells is granulated because the thin micro- [illegible] ...gration. [illegible] ...he cells.



Fig 4 A more coarse irregular surface which relatively often occurs on nasal polyps On the other hand, it is unusual to see so many cytoplasm protuberances (*arrows*) from the epithelial cells × 1 680

RESULTS

The ultrastructure of the mucous membrane on the inferior turbinate has been described previously (Mygind & Bretlau, 1973 and 1974) In this paper we shall mainly describe structures which are characteristic of nasal polyps and regarding description of squamous epithelium, cilia, microvilli, goblet cells and other structures we must refer to our previous publications

All polyps gave specimens fit for use The epithelial surface was practically free of secretion, and only a few glandular openings were noted The appearance of the surface differed considerably from one polyp to the other and from place to place on the same polyp Thus it could be observed that while the anterior surface of a polyp was covered entirely by microvilli, the posterior surface was totally covered by cilia

The type of epithelium varied from a pseudostratified cylindrical, to a squamous type, with intermediate forms in between Pseudostratified cylindrical epithelium was most frequent, and in only three SEM specimen could a typical squamous epithelium be observed The epithelium of the polyps showed variation in thic ness and cell composition in the same the mucous membrane on the inferio A characteristic low epithelium of diate type, resembling transit

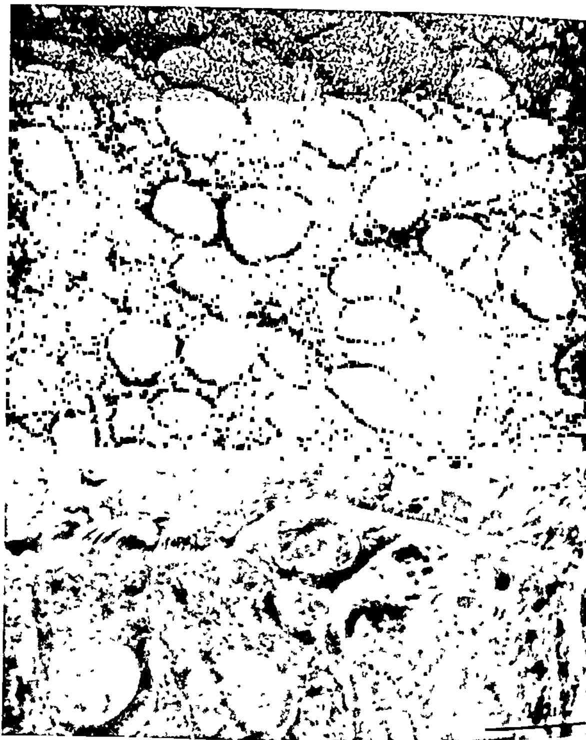


Fig 5 Typical for nasal polyps, an extraordinarily great variation in the surface area of the epithelial cells, observed particularly in the presence of very large cells The surface is covered by microvilli but a single group of cilia are seen, corresponding to one cell (*arrow*) ×1 680

Fig 6 Very occasionally, flat cells can be seen lying on top of the normal epithelial cells This photo is from one of the rare glandular ducts, the surface is therefore covered with secretion Original magnification ×800.

found in the urinary bladder, was seen in several places By means of SEM, the surface-cells in this epithelium appeared box-shaped and protruded, dome like at the surface, giving a general appearance of cobble stones (Figs 1 and 2). The surface relief with the individual cells can also vary considerably Besides those cells looking like cobble-stones, it is also possible to observe an even surface, where the cells are found as flagstones in a mosaic (Fig 3*a*), or an irregular prominence of the individual cells (Fig 3*b*), or finally, a picture with defective terminal bars and widened intercellular spaces (Fig 3*c*) The last mentioned phenomenon could be seen in one or several places in half of the specimens This corresponds approximately to the conditions on the inferior turbinate in patients with perennial rhinitis The cells resembling cobble stones are, however, characteristic of polyps (Fig 1) and the occurrence of an irregular surface with an appearance resembling a hilly landscape in which every unevenness consists of several cells (Fig 4)

The cell borders are most often visible and slightly depressed The surface shape of the cells is often round, but may be polygonal (Fig 3*a*) It is very characteristic to see a great variation in the surface area of the cells with the occurrence of large cells with a surface area of up to 150 μm^2 (Fig 5) Most of these cells are, in anoptral contrast, found to be the previously described large box shaped cells in a transitional epithelium, but in a few places it is possible in a typical pseudostratified cylindrical epithelium to see single flat cells without contact with the basement membrane, lying on top of the normal epithelial cells (Fig 6)

Filled goblet cells could be identified, but cells being emptied were seldom seen The same applied to cytoplasm protuberances from the epithelial cells (these formations are also called apocrine secretion) which were found in a considerably lower number than on the inferior turbinate Now and then cells could be seen penetrating the epithelium (Fig 1) These cells are probably eosinophil leucocytes

On the polyps, microvilli have the same appearance and are found to the same extent as on the inferior turbinate The same applies to cilia, which cover about 30 % of the total surface of the polyps It should be noted that visible destruction of the cilia or signs of increased ciliogenesis was not observed in one single specimen It is, however, impossible to say what the normal rate of ciliogenesis is in the mucous membrane in the upper part of the nasal cavity We have not been able to collect suitable normal references from this area, but have only succeeded in obtaining two specimens from the upper part of the respiratory nasal mucosa in patients undergoing ethmoidectomy on account of sinusitis In these specimens ciliogenesis was not observed The surface was covered with normal-looking cilia, which appeared slightly longer than the cilia described at the anterior part of the inferior turbinate (Mygind & Bretlau, 1973) However, further investigations are needed to confirm this observation

DISCUSSION

Nasal polyps are most suitable for SEM investigations, as a large untouched and secretion free surface can be obtained for microscopy without any kind of anaesthesia However, it is important to choose a surface which has not been pressed against the mucous membrane of the nose or against another polyp The microscopy is greatly facilitated because there is almost no mucus on the surface at all This is undoubtedly, due to the small number of glands, the contribution of gravity in removal of mucus and the influence of the inspired air on the mucous membrane, which is thereby transformed in the direction of the mucous membrane in the front of the nasal cavity

The surface of nasal polyps does not differ essentially from the surface of the inferior turbinate in patients with perennial rhinitis This has been described in previous pub
(Mygind & Bretlau, 1973 and 1974
of the epithelium as the polyp
ume may be the reason why

of *transitional* type is more often seen in the polyps The epithelial cells are often dome-shaped apically, which Lenz (1972) *also has* observed on the *inferior turbinate* in patients with perennial rhinitis This phenomenon is most easily explained as a result of intracellular oedema However, we have *not observed the* very pronounced degrees of intra- and intercellular oedema, as described in the transmission electron microscope study of Jahnke (*1972*) *In* half of the specimens *the intercellular* space between the epithelial cells was widened in a single part or in the whole of the *biopsy It must* not be forgotten, however, that *quite pronounced* changes *take place in* a biological material when it is fixed, dehydrated, freeze-dried and metal-coated If it was the case, *in vivo too,* that the intercellular *spaces were* greatly widened and the terminal bars quite defective, *this* would mean that the large *amounts* of tissue-fluid in general would be *free to diffuse* through the epithelium, *as the terminal* bars are the most effective barrier against such a *transport* (Terrahe & Backwinkel, 1970) *It is not* in accord- nce with common *clinical* experience to see rous secretion of any importance from nasal polyps, since the latter *would, in that* case, spontaneously diminish Several *papers have unanimously* shown that epithelial cells and pieces of epithelium are discharged in increased *quantities* in allergic mucous membranes (Mygind & Thomsen, 1973, Naylor, 1962, Sanerkin & Evans 1965) *The most* obvious explanation of the above-mentioned phenomenon is therefore that in the areas in question, a *certain intercellular* oedema is present as *well as* reduced *cohesion* between the cells Shrinkage during preparation augments these *tendencies and* the specimens appear in SEM *with greatly* increased *intercellular spaces and* entirely defective *terminal* bars The phenomenon observed must *therefore be a structurally* dependent artifact *and, as such*, a valuable *observation*

The most striking new *information* obtained by the *SEM investigation* of nasal polyps, is that the surface-area of the epithelial cells can vary considerably It is now *known what differences there* may be between small and large *cells regarding* age and function

It was an important *observation that the cilia* on *nasal polyps had* a quite normal appearance *It is therefore* most likely that the destruction *of cilia, which* we previously have observed *on* the inferior *turbinate in* patients with perennial *rhinitis, is due* to the fact that these cilia are *exposed to* pressure from an increased amount of mucus or from the nasal septum, with *which* the *swollen turbinate* temporarily comes into *contact It* may be concluded that in the chronic allergic mucous membrane, no signs are found of the specific destruction of cilia which has been *described in sensitized* animals, exposed to *grass-pollen* (Chevance, 1971)

The changes we have found *on* the surface of nasal *polyps were of* such a character that there *is* no reason to assume that they are specific of polyps of allergic genesis This assumption is confirmed by the fact that it was impossible to detect *any differences* between the surface of the polyps from patients with positive, and from patients with negative allergological examination

ACKNOWLEDGEMENTS

The Cambridge Stereoscan microscope was kindly placed *at our disposal* by the Institute for Historical Geology and Palaeontology (H J Hansen M Sc) We owe Mr Jorgen Fuglsang great thanks for operating the microscope for us Gratitude is also expressed to Mrs Bente Christensen and Mr Erik Jensenius for skilful technical assistance

ZUSAMMENFASSUNG

Nasenpolypen konnten gut im Rasterelektronenmikroskop untersucht werden Es wurde eine geringe Anzahl Drusen und Protuberanzen des Zytoplasmas der Epithelzellen gefunden 30 % der totalen Oberfläche war von Zilien ohne Anzeichen einer Destruktion bedeckt Die Hälfte der Praparate wies Gebiete mit erweiterten Interzellularräumen auf Bei Patienten mit vasomotorischer Rhinitis unterschied sich die Oberflache der Polypen kaum von der der unteren Nasenmuschel Es war aber charakteristisch dass beiden Polypen Zellen eines Übergangsepithels wie Pflastersteine kuppelformig hervorragten Ausserdem wurde bei Zellen bis zu einer Oberflächengrösse von 150 μm^2 eine grosse Variation des Oberflächeninhalts beobachtet. Eine andere charakteristische Erscheinung war eine un-

ßene Oberflache, einer hügeligen Landschaft ähnlich, ßren Unebenheiten jeweils aus mehreren Zellen bestanden

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THE INFLUENCE OF TEMPERATURE ON MUCOCILIARY ACTIVITY

Temperature range 20°C–40°C

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(Received January 24 1974)

Abstract In vitro experiments have been performed on mucous membranes from rabbit tracheae in order to investigate the influence of temperatures between 20 and 40°C on the mucociliary activity

The following results have been obtained

1 The mucociliary activity including mucociliary wave frequency and coordination of wave patterns is most regular at or near the body temperature

2 From body temperature to room temperature a 50% reduction of the wave frequency was demonstrated together with irregular changes in frequency and amplitude

3 The effect of temperature alterations was reversible hin at most 3 hours

4 The relationship between mucociliary wave movements and temperatures between 20° and 40 C looked practically linear contrary to most temperature dependent physiological processes This indicates that more than one factor is involved in the surface light reflections e g the intracellular activity and mucus rheology

Temperature influences all biological processes including the function of the mucociliary system of the respiratory tract Experimental investigations illustrating this subject have been published by many investigators using different methods The transport velocity in mammals has been studied by among others Dalhamn (1956), Hill (1957) and Tanaka (1967) These authors found a maximum effect of ciliary transport at a temperature of 36–40 C The "ciliary beat frequency" in mammals has been investigated by Ballenger & Orr (1963) and by Iravani (1967) using stroboscopy and also by Proetz (1934), Dalhamn (1956, 1960) and Iravani & Stein hausen (1967) with the use of cinematographic methods (Table I) Tanaka (1967) has compared the "ciliary beat frequency of rabbit tracheae at different temperatures in vivo by a photo-electric technique He found maximum ciliary function at about 30°C

This investigation has been supported by grants from the Swedish Medical Research Council Project number B73 14X 3897-01

The use of different experimental animals and a number of different recording techniques makes it impossible to compare the cited investigations. Furthermore, a review of the literature failed to reveal any investigations based on a systematic study of graduated increases and decreases of the temperature parameter within the range of 20° to 40°C

The mucociliary system of the respiratory tract may be influenced by temperature changes during febrile illnesses and also as a result of hypothermia as utilized in special types of anaesthesia The ciliary function may also be influenced by the temperature of the inspired air if the physiological air conditioning of the nose is bypassed

In view of this clinical background we considered that a special survey of the temperature parameter might be of theoretical and practical importance Furthermore, it is necessary to know the effect of temperature changes on mucociliary activity in the temperature range between room temperature and body temperature, since many applied experimental studies on cilia are made on animals in the laboratory environment

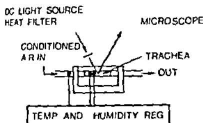


Fig 1 Experimental arrangement for indirect recording of mucociliary wave patterns *via* surface light reflections in an air-conditioned experimental chamber

METHOD AND MATERIAL

A standardized method for the study of mucociliary wave movements has already been published (Mercke et al, 1974, Toremalm et al, 1974) Mucociliary wave movements are recorded indirectly via surface light reflections in vitro

After thorough preparation, part of a rabbit trachea was mounted in a special experimental chamber, as illustrated in Fig 1 A light beam was directed onto the tracheal mucous membrane through a slit made in the pars membranacea The variations of light intensity picked up by the microscope are transformed into electrical voltages and amplified by the photomultiplier and recorded by an ink writer The temperature of the tracheal specimen and the air blown through it in one direction were monitored by four thermocouples Two of them (ELLAB type KCl) were placed within the tracheal lumen for control of the air temperature and two on the outside of the tracheal wall

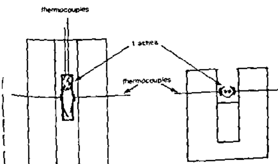


Fig 2 Cross section of the experimental perspex chamber containing the tracheal specimen The sites of intra tracheal and extratracheal thermocouples are shown

(ELLAB type HI) for control of the tissue temperature, as seen in Fig 2

The investigation was comprised of eleven experimental sequences with tracheal specimens from 6 rabbits with a weight of 1–1 5 kg The animals were killed with a blow on the head in order to avoid pharmacological side effects The relative humidity was kept between 90–100 %, which was controlled by a psycrometer (ELLAB type B19)

The recordings were analysed and the average wave frequency determined for periods of 60 sec In addition, the frequency spectrum of the mucociliary wave movements was analysed by computer

RESULTS

A summary of all results is given in Table II The results from one experimental sequence are shown in Fig 3 A gradual increase of temperature from 21°C to nearly 40 C during 85 min resulted in an increase of the mucociliary wave movements from about 420 to about 1 000 waves/min Increased temperature also brought about a more uniform rhythm With a subsequent decrease in temperature to 19 1°C during 197 min the frequency of the wave movements was reduced to 360 waves/min and the rhythm became more irregular again with a great number of variations in amplitude and frequency

Fig 4 shows another experimental sequence in which the temperature was increased from 20 to 40 C followed by a subsequent decrease to 20 9 C The temperature was then once again increased to 40 3°C This experimental sequence lasted 313 minutes A maximum uniformity of the mucociliary wave movements was observed at 35–40 C

The results from three different temperature ranges in Fig 3 were also processed by computer (Fig 5) As the temperature increased from 20 9 to 39 9 C, the frequency of mucociliary wave movements also increased [illegible] to 20 waves/sec The scatter is about [illegible] sec at each of these temperatures

All the results regarding [illegible] and mucociliary activity [illegible]

Table I

S = Stroboscopical methods C = Cinematographical methods P = Photoelectrical method

Author	Method	Animal	In vitro	In vivo	Temp range (°C)	Freq range (beats min⁻¹)	Max frequency at (°C)
Proetz, 1934	C	Rabbit		×	5–50	0–720	18–33
	C	Homo	×		5–50	0–720	18 33
Dalhamn 1956	C	Rat		×	37–41	1 097–1 290	41
Dalhamn 1960	C	Rabbit		×	33 9–38 6	911–1 404	38 6
Iravani Steinhausen, 1967	C	Rat	×		4–25	10–264	—
Ballenger Orr, 1963	S	Guinea pig	×		C 20–38	850–1 600	—
Iravani, 1967	S	Rat	×		4–44	—	38–40
Tanaka, 1967	P	Rabbit		×	20–40	C 1 030–1170	C 30

have also been plotted graphically The frequencies of mucociliary wave movements were relatively well concentrated along a regression line (Fig 6) The frequency/temperature relationship is not strictly logarithmic or linear The regression line flattens out slightly at temperatures at and above body temperature

DISCUSSION

The mucociliary function of the respiratory tract is influenced by the body temperature and may also be affected by the temperature of the inspired air This relationship has long ago been observed by many investigators using stroboscopic, cinematographic or photoelectric methods (Table I) The results obtained during a period of four decades, vary greatly Cilia from different mammalian tissues have been investigated experimentally under various degrees of control The maximum frequency was found within a limit of 18° to 41°C As far as we know there has been no investigation based on continuously increasing and decreasing temperatures between 20° and 40°C with satisfactory simultaneous control of the relative humidity of the surrounding air It has not therefore been possible to draw any definitive conclusions from the

Table II

I = Temperature increment
D = Temperature decrement

		Initial temperature (°C)	Final temperature (°C)	Experimental duration (min)	Mucociliary wave movements (waves/min)	Number of observations
Rabbit no 1	D	40 4	21 1	165	1 034 → 292	16
Rabbit no 2	I	21 0	40 3	85	420 → 1 020	11
	D	40 3	19 1	197	1 020 → 360	16
Rabbit no 3	D	35 0	20 1	108	893 → 345	9
	I	20 1	38 3	72	345 → 1 007	8
Rabbit no 4	I	23 8	40 9	87	428 → 751	10
	D	40 9	27 0	135	751 → 509	9
Rabbit no 5	I	21 1	40 2	81	390 → 972	11
Rabbit no 6	I	19 9	40 1	61	341 → 779	8
	D	40 1	20 9	191	779 → 295	10
	I	20 9	40 3	61	295 → 843	7

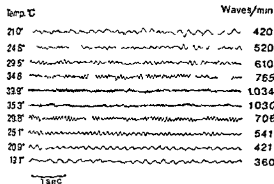


Fig 3 Recordings and average mucociliary wave frequencies from one tracheal specimen at various temperature intervals between 19 1° and 39 9°C

literature quoted regarding the temperature influence on mucociliary activity in the respiratory tract

Our standardized method for experimental in vitro studies of cilia is described in a previous paper (Mercke et al, 1974) It is also necessary to standardize the temperature and humidity parameters during applied experiments, for example where respiratory cilia are used to test the influence of inhaled gases and particles For laboratory purposes the temperature range between 20° and 40°C—representing ordinary room temperature to body temperature—is of most importance and interest

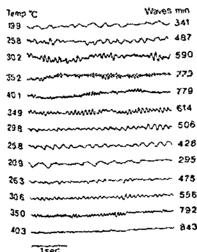


Fig 4 Recordings and average mucociliary wave frequencies from one tracheal specimen at various temperature intervals between 19 9 and 40 3 C

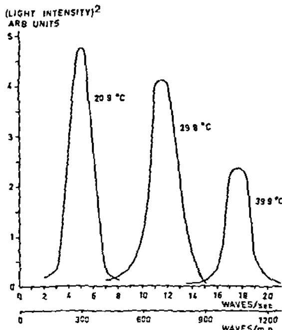


Fig 5 The relationship between mucociliary wave frequency at three different temperatures and reflected light intensity in the sequence also shown at Fig. 3 At each temperature, the frequency varies by about ±1 wave/sec

In the recordings shown in Fig 3 the mucociliary wave frequency is about 1 000 waves/min at body temperature The rectal temperature of afebrile rabbits is at most 39°C according to Altman & Dittmer (1966) In the present investigation we have found that a temperature reduction from 40° to 20°C is accompanied by a decrease of the mucociliary wave frequency of about 50 %

According to the very few actual experiments available (Table 1) the difference between in vivo and in vitro experiments seems to be about 200 waves/min There are, however, many arguments in favour of in vitro experiments For example, such disturbing factors as interference from heart- and respiratory movements and alternating air flow directions, as well as the influence of anaesthetics, can be excluded However, the rhythmical contractions of the smooth muscles of the tracheal wall may also be observed in in vitro experiments (Håkansson & T malm, 1967)

As can be seen from the surface r

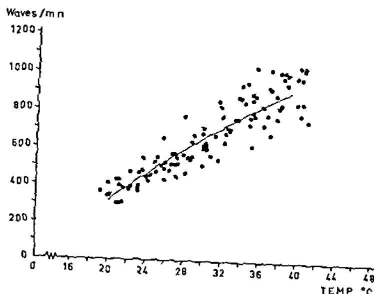


Fig 6 The relationship between all wave frequency averages and temperatures in the range 20–40 C (cf Table II) for the 11 experimental sequences

shown in Figs 3 and 4 the frequency and amplitude of the reflections are not the only points of interest There is also a more or less regular rhythm of amplitude and frequency variations

Since the reflections are dependent on mucous surface waves as well as on moving cilia (Toremalm et al, 1974) an incongruence may appear in the two reflection points At 35°–40°C the rhythm is very regular which can be due to addition of the two reflections without any appreciable phase shifting At lower temperatures (20°–30°C) the cilia are presumed to work with a reduced continuous frequency and the mucous waves may be retarded due to increased tenacity While the mucous ridges are lowered, giving less light reflection, the cilia are at the same time sweeping more slowly, giving an extended period of reflection The recordings at 20°C are therefore irregular, in addition to the reduction of mucociliary wave frequency and decrease of secretional wave amplitudes

The distributions of average wave frequencies at three temperatures from the sequence shown at Fig 3 have been processed by computer The result appears at Fig 5 From this the mean frequency and scatter at each temperature can be easily seen The scatter is only about ±1 wave/sec at all three temperatures This speaks in favour of a regular basic frequency produced by the cilia The total number of results obtained from the trachea of all six rabbits are shown in Table II We have not found any signs of hysteresis in the sequences with successively increases and decreases of temperature

All results have been pooled in a frequency/temperature diagram (Fig 6) From this it is obvious that the frequency of reflections increases with increasing temperature, following a regression which is virtually linear The range of dispersion between the animals is about 100 waves/min at 20 C and 300 waves/min at 40 C This curve differs from the usual pattern of biological processes, where a rise in temperature is generally combined with an exponential increase of activity Guttman (1969) for example has shown increase in Q_{10} values for repetitive responses in squid axons following increased temperatures For surface tension forces however, the temperature dependency is strictly linear (Adamson, 1967) In the present investigation the Q_{10} value was 2 1 between 20 and 30°C and 1 4 between 30° and 40 C It is most plausible that more than one parameter is involved in the present results summarized in Fig 6 We know some of them One is the intracellular "pacemaker", another the tenacity of the secretion and a third the surface tension In the present case, the tenacity and surface tension forces are dominant at low temperatures, so that an exponential increase cannot

e expected It should therefore be very interesting to study the ciliary wave frequency without he mucous cover However, such changes of he mucous layer are not possible without impairing physiological functions (Sade et al, 970)

The present results do not allow any direct conclusions regarding the influence of temperature on mucociliary function from a clinical point of view The difference between the function of human and mammalian respiratory cilia is very small (Proetz, 1934) as is also the difference between in vivo experiments and tracheal in vitro preparations taken immediately post mortem A functional reduction of mucociliary activity is therefore probable e g in patients during hypothermic anaesthesia A far more interesting problem is the mucociliary transport mechanism in febrile patients The etiology of bronchopneumonia and atelectasis especially needs to be studied from this point of view Regarding the possible influence on cilia of untempered inspired air, there are no problems during physiological ventilation thanks to the excellent heat and moisture exchange function of the nose and larynx It has been found that during nasal breathing at a room temperature of 21°–27°C the inspired air was 32 34 C when it passed the nasopharynx (Cole, 1953) Ingelstedt (1956) found a temperature of the inspired air in the subglottal space of 32 3°C during nasal breathing and 30 5°C during oral breathing The difference is due to the intimate contact between the turbulent air stream and the mucous membrane made possible by the shape of the nasal cavities Ingelstedt & Toremalm (1960) found an air temperature of 25 C in the nasopharynx of a patient exposed to an environmental temperature of 12 C The inspired air is therefore well tempered when it passes through the trachea during normal breathing and does not greatly influence the mucociliary function

The present basic laboratory study gave results which should be considered as showing that standardized recording methods are in themselves inadequate The temperature parameter must be kept under control in applied studies into the influence of pharmacological and toxicological substances on mucous membranes

ZUSAMMENFASSUNG

Der Einfluss von Temperaturen zwischen 20 and 40 C auf die mukoziliare Aktivitat ist vermittels *in vitro* Versuchen an der Trachealschleimhaut von Kaninchen untersucht worden

Es ergaben sich folgende Resultate

1 Die mucoziliare Aktivitat einschliesslich der mucoziliaren Wellenfrequenz und der Koordination des Wellenmusters ist am regelmassigsten bei oder in Nähe der Körpertemperatur

2 Bei Senkung der Temperatur von Korpertemperatur auf Zimmertemperatur konnten eine 50prozentige Herabsetzung der Wellenfrequenz sowie unregelmassige Frequenz und Amplitudenveranderungen festgestellt werden

3 Der durch Temperaturveranderungen hervorgerufene Effekt war innerhalb von hochstens drei Stunden reversibel

4 Im Gegensatz zu den meisten temperaturabhangigen physiologischen Prozessen erwies sich das Verhältnis zwischen den mukoziliaren Wellenbewegungen und Schwankungen der Temperatur zwischen 20 und 40 C als fast linear Dies Resultat deutet darauf hin dass mehr als ein Faktor die Lichtreflexe an der Schleimhautober flache hervorruft z. B die Intrazellularaktivität und Schleimrheologie

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THYROPLASTY AS A NEW PHONOSURGICAL TECHNIQUE

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bstract In an attempt to examine the surgical possibility
' changing the vocal cord position and tension by re-
rming the thyroid cartilage, an experimental study was
ade using 10 adult dogs Hoarseness produced by sec-
[illegible]

oint Their effects on the vocal cord are (1) lateral com
ession, (2) lateral expansion (3) relaxation (shorten
g) and (4) stretching (lengthening) respectively Pos
ble indications for each type of thyroplasty were de-
ribed with reference to specific laryngeal diseases The
lvantages of thyroplasty were emphasized namely, that
e intervention inside the thyroid cartilage is minimal
id therefore fine and reliable adjustment is possible
iring surgery Thyroplasty thus offers a new possibility
phonosurgery

he aim of treatment for laryngeal diseases is ιost frequently to attain a normal voice, although this becomes secondary in such cases s laryngeal carcinoma or bilateral recurrent ierve paralysis Phonosurgery, defined as surgery for better voice, is at present almost limited o removal of a benign tumor on the vocal cord or reduction of the glottal chink by teflon injection or cartilage implantation for unilateral recurrent nerve paralysis Despite the recent development of laryngo-microsurgical technique, direct intervention on the vocal cord itself, z-plasty for sulcus vocalis for instance, has not yet been performed

There may be several factors which prevent direct surgical intervention on the vocal cord Surgical techniques which can be used through a suspension laryngoscope are naturally limited and fine suturing as is necessary for z plasty is virtually impossible Laryngofissure, which would then be required for such a technique, poses another unsolved problem—postoperative development of granuloma at the anterior commissure The greatest obstacle we encounter, however, is the inevitable postoperative scarring of the mucosa, which reduces the compliance of the vocal cord mucosa, thus greatly hindering vibration of the vocal cord

As a result, phonosurgery at present is quite incompetent for laryngeal diseases such as mutational voice disorders, hyperfunctional dysphonia, vocal cord hypertrophy due to anabolic hormones, sulcus vocalis, vocal cord atrophy, and traumatic laryngeal deformities We laryngologists should thus break away from conventional vocal cord polypotomy As a stage prior to direct surgical intervention on the vocal cord, we attempted to change the position and physical property of the vocal cord by actively reforming the cartilaginous frame-work on which vocal cords are suspended

A search of the literature fails to reveal any similar systematic investigation on how the reformation of the thyroid cartilage affects the vocal cord and the voice This preliminary report deals with the experimental results of thyroplasty in dogs Several clinical cases will be reported in detail later

EXPERIMENTAL PROCEDURE

Ten adult dogs were used for the experiment The dogs were anesthesized by intraperi injection of Pentobarbital Natrium (Mi mg, and fixed supine for laryngeal

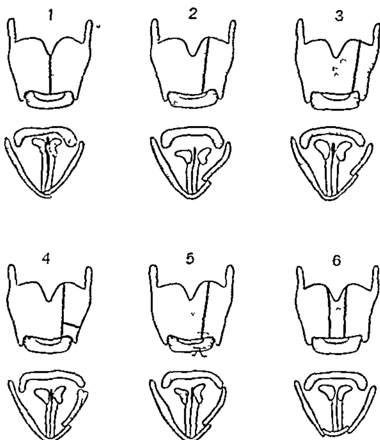


Fig 1 Schematic demonstration of varied thyroplasties performed on dogs

local anesthesia with 1% Xylocaine, the nx and left recurrent laryngeal nerve were exposed For better visualization of the vocal cord, supraglottic laryngectomy was performed Voicing was evoked by pain stimuli For each experimental condition mentioned below, laryngeal pictures—black and white, color photographs, and 16 mm cinefilm—were taken during respiration and phonation The voice was recorded by tape-recorder for later acoustic analysis Before sectioning of the recurrent laryngeal nerve, the vocal cord movement and voice were confirmed to be normal Any dog whose respiratory movement of the vocal cord was asymmetrical was excluded from the analysis of the experimental results

After section of the left recurrent laryngeal nerve, thyroplasty was performed vertical incision of the left lamina was made at 1 the median line, 2 anterior one third, 3 middle, and the lateral part of the cartilage lamina was slipped underneath the medial part and fixed by 4-0 Nylon suture The two laminae were overlapped 2–4 mm width depending on the case In addition to the recurrent laryngeal nerve the external branch of the left superior laryngeal nerve was also cut in two dogs Two parallel vertical incisions on the thyroid cartilage and depression of the median cartilage were done in one dog Incisions and fixation of the thyroid cartilage used in the present experiment are schematically shown in Fig 1

EXPERIMENTAL RESULTS

1 *Median incision on thyroid cartilage and repositioning of left lamina (1 in Fig 1, Dog no 1)*

The hoarse voice caused by section of the left recurrent laryngeal nerve was somewhat improved by the above procedure but still re-



2 *Left* control, during phonation, *middle* after ...tion of the left recurrent laryngeal nerve, during phonation, *right* after thyroplasty during phonation The arrow indicates the site of repositioning

...ined rough The procedure resulted in the ...ft of the paralysed vocal cord slightly toward ... midline, with the decrease of glottal slit ...ring phonation Nevertheless a noticeable ...nk remained at the posterior glottis (cartilagi...us portion) The voice was lowered in pitch ...slipping in the left lamina

2 Vertical incision of left lamina at anterior middle third and shift of lateral (posterior) lamina underneath the median (anterior) lamina (2 in Fig 1, Dogs 2, 4, 7, 8, 9, 10)

...oarseness due to recurrent nerve section was ...markably improved, particularly in dogs 4 ...d 9, whose voices became quite normal (Fig ... In general, there remained a narrow slit at ... cartilaginous portion The vocal pitch did ...t change markedly In dog no 8, the inner ...richondrium was separated over a wide area ...om the thyroid lamina along the vertical in...on line so that the lateral part could be slipped ... anteriorly underneath the median part ...is wide perichondrium separation and over...oping of the laminae resulted in prominent ...lging of the vocal cord, and the voice re...ained hoarse and low-pitched The excessive ...crease in thickness seemed to have arrested ... vibration of the vocal cord

3 Vertical incision at antero-posterior mid line and shift of lateral part (3 in Fig 1, Dog no 3)

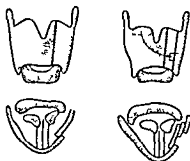
The hoarseness was not much improved by this procedure A slit remained not only at the cartilaginous portion but also at the anterior glottis

4 Vertical incision at anterior middle third supplemented by horizontal incision and shift of upper lateral part (4 in Fig 1, Dog no 2)

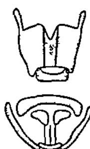
A slit always remained at the cartilaginous portion even following the procedures 1–3 in Fig 1 It was assumed that the inferior horn or cricothyroid joint was in the way of the lateral lamina shifting medially For this reason, an additional incision was placed horizontally above (cranially) the cricothyroid joint to shift the posterior part of the lamina more medially

This tilted T-shaped incision (|—) unexpectedly ineffectuated the function of the cricothyroid muscle, because the muscle attachment on the thyroid cartilage was indirectly disconnected with the anterior commissure Consequently the vocal cord was shortened and slightly abducted The slit did not become narrower and the resultant voice improvement not remarkable

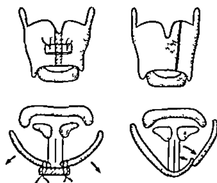
Type I : Lateral Compression



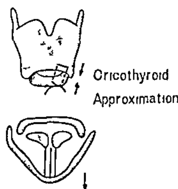
Type III : Shortening



Type II : Lateral Expansion



Type IV : Lengthening



3 Four types of thyroplasty

5 Vertical incision at anterior middle third and anterior approximation of cricothyroid distance (5 in Fig 1, dogs 6 and 9)

The recurrent laryngeal nerve and the external branch of the superior laryngeal nerve on the left were cut first The consequent glottal chick during phonation was not sufficiently eliminated by mere vertical incision at the anterior middle third of the thyroid lamina The difference in level between the bilateral cords remained, with the left cord positioning higher than the right The left cord was so lax that it vibrated only at the initial stage of phonation but soon remained open without vibration The voice which started with a rough quality, soon became breathy with a widening glottal chink during phonation

Then, the cricoid cartilage was approximated to the thyroid by 4-0 Nylon suture just as in the case of a contraction of the cricothyroid muscle The result was excellent the voice was improved almost to the normal with only a slight posterior glottal slit (dog no 6) or without any slit (dog no 9)

6 Parallel vertical incision on thyroid cartilage on each side of midline and about 1 cm from it (6 in Fig 1, dog no 5)

In this case, the recurrent laryngeal nerve was not sectioned After the parallel incisions, the median segment was depressed to slip in The voice was much lowered in pitch but not hoarse Pulling up and fixing the median segment back in the original position resulted in a rise of pitch up to the previous level

DISCUSSION

The results of the present experiment indicated that re-formation of the thyroid cartilage can quite effectively modify the position and tension of the vocal cord Clinical indications for the techniques may therefore include insufficient glottal closure or excessive tension of the vocal cord Treatment of dysphonia due to unilateral vocal cord paralysis, which seems most relevant to the present thyroplasty is reviewed below to evaluate the technique

1 *Surgical treatment of dysphonia due to unilateral vocal cord paralysis*

In 1911, Brünings first developed a method of narrowing the glottis by paraffin injection in case of a unilateral recurrent nerve paralysis Despite his success, this method had long been abandoned, probably because of the side effects such as paraffinoma Recently, Arnold (1962 and 1964) and many others revived this approach with new synthetic materials such as teflon, tantalum powder, and silicone

In 1915 Payr utilized a tilted U shaped incision on the thyroid ala to make a pedicled cartilage flap, which was then depressed inward to shift the vocal cord medially Probably because of the pedicle, the shift effect was limited and the technique did not gain popularity

It was Meurman (1952) who first utilized cartilage implant for this purpose The implant taken from the costal rib was inserted between the thyroid cartilage and the inner perichondrium Opheim (1955) used an incised segment of the thyroid cartilage instead, which was inserted inside the inner perichondrium at the level of the vocal cord Direct intervention on the soft tissue just lateral to the vocal cord gave rise to postoperative edema, eventually requiring tracheotomy Sawashima et al (1968) inserted an incised segment of the thyroid cartilage between the thyroid ala and inner perichondrium, with excellent results in voice Kamer & Som (1972) inserted a piece of the thyroid cartilage from the lower rim of the thyroid cartilage in case of traumatic vocal cord paralysis

An experimental attempt was made by Bernstein & Holt (1967) to reposition the vocal cord by inserting the bipedicled vital sternohyoid muscle between the thyroid cartilage and inner perichondrium Transposition or rotation of the arytenoid cartilage (Morrison's reverse King operation, 1948, and Montgomery's Arthrodesis, 1966) for this purpose does not seem very popular because of its technical difficulty Mündnich (1970) pulled and fixed the arytenoid toward the lower horn of the thyroid cartilage without fenestration of the thyroid cartilage for tensing and median shifting of the vocal cord

At present, injection of various synthetic materials appears most popular as the treatment of hoarseness in unilateral recurrent nerve paralysis Percutaneous injection methods were recently reported by Hurst (1972) and Sato et al (1971)

As Bernstein & Holt (1967) described, there seem to be many problems left unsolved in the above treatments the possibilities are neoplastic response or eventual rejection by the tissue of the synthetic materials, migration or absorption of the implanted tissue, local stiffening of the vocal cord due to injection and so on

On account of the advantage that our procedure is rather simple and easy to perform and does not intervene inside the thyroid cartilage, the thyroplasty seems to have a place in treating dysphonia of vocal cord paralysis in man

2 *From the functional view point thyroplasty (Isshiki) can be classified into the following 4 types (Fig 3)*

Type I Lateral Compression

This type would be of widest application Depending on the antero-posterior location and the degree of the glottal chink, incision and overlapping can be adjusted rather anterior incision may be used for anterior chink, and a wedge may be inserted between the overlapping cartilage laminae to reinforce the median shift of the vocal cord An added horizontal incisi or rectangular incision may also be a f possibility to be considered

Type II Lateral Expansion

There would be few indications for this type of procedure It also seems rather difficult to widen the angle of the thyroid cartilage Indications for this type in hyperfunctional dysphonia such as dysphonia plica ventricularis would be a future problem to be examined

Type III Relaxation (Shortening) of Vocal Cord

Too high pitch as in mutational voice disorder or sulcus vocalis may be a good indication for this type of thyroplasty Niimi et al (1973) already reported that a square incision around the anterior commissure and depression of the segment was effective for treating dysphonia in sulcus vocalis

Type IV Stretching (Lengthening) of Vocal Cord

We attempted to lengthen the thyroid cartilage horizontally by squeezing a cartilage segment into the vertical incision crevice, but failed For this purpose, approximation of the anterior …othyroid distance by mattress sutures was … easier and more effective Possible late …sening of the knot is a problem worthy of … consideration

Mixed types of the above are naturally possible Actually, we clinically adopted a mixed type I and III for mutational voice disorder and I and IV for combined paralysis of the recurrent laryngeal nerve and the external branch of the superior laryngeal nerve The details of the clinical cases will be reported later but the advantage is that we can select the type and adjust the extent of reformation during operation by the produced voice quality

3 *Limitation of present experiment*

The cartilaginous portion of the vocal cord is relatively much longer in the dog than in man It is therefore assumed that the deficient glottal closure at the cartilaginous portion remaining after thyroplasty may be less important in the human case Present experiments are all acute In clinical cases of vocal cord paralysis, further consideration may be required on the problem of accompanying vocal cord atrophy or compensatory movement of the intact vocal cord However, the present experiment provided a fair prospect of clinical application of thyroplasty

Together with metrological study on the human cadaver larynx now under way, clinical application has already been started Thyroplasty as we proposed offers a new possibility in phonosurgery

ZUSAMMENFASSUNG

Die Frage, ob sowohl die Stellung als auch die Spannung der Stimmbänder durch die Umgestaltung des Schild knorpels verändert werden konnen, wurde anhand von experimentellen Tierversuchen mit 10 erwachsenen Hunden untersucht Durch die vertikale Abschneidung des Schildknorpelflugels und die mediale Verlagerung seines lateralen Stuckchens wurde die mittels Durchtrennung des N recurrens hervorgerufene Heiserkeit im allgemeinen ausgezeichnet verbessert

Vom funktionellen Standpunkt aus wurden 4 Typen von der Thyreoplastik vorgeschlagen die jeweils folgende Wirkung auf die Stimmbänder haben 1 Laterale Kompression 2 Laterale Expansion 3 Erschlaffung (Verkurzung) 4 Spannung (Verlangerung) Ihre Operationsindikation wurde fur jede Kehlkopfkrankheit erstellt Als Vorteile der Thyreoplastik beurteilen wir dass der operative Eingriff in das Innere des Schildknorpels *minimal ist, und dass daher die feine* und sichere Regulierung intraoperativ ohne weiters durchgefuhrt werden kann

Die Thyreoplastik setzt dehalb ihre grosse Hoffnung auf die Phonochirurgie

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EXTRACARDIAC RHABDOMYOMA

Light and electron microscopic studies of two cases in the mandibular area, with a review of previous reports

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Abstract Two cases of rhabdomyoma (one of them multifocal) in the submandibular region are described Electron microscopic investigation of one case revealed rudimentary myofibrils which in some places showed a random arrangement, while in other parts they formed stellate figures Hypertrophic Z bands were seen, as also were occasional A and I bands The pathology and clinical features of rhabdomyoma are discussed on the basis of these cases and those to be found in the literature As extracardiac rhabdomyoma is most commonly found in the region of the head and neck, it is recommended that this tumour, rare though it is should been borne in mind in the differential diagnosis of tumours in this region

The term rhabdomyoma is used for two types of tumour, the one cardiac and the other the far rarer extracardiac form which according to Moran & Enterline (1964) differs in its morphology Extracardiac rhabdomyoma is considered to be a benign neoplasm of striated muscle, whereas the cardiac form is often associated with tuberous sclerosis and is more probably a developmental anomaly (Moran & Enterline, 1964)

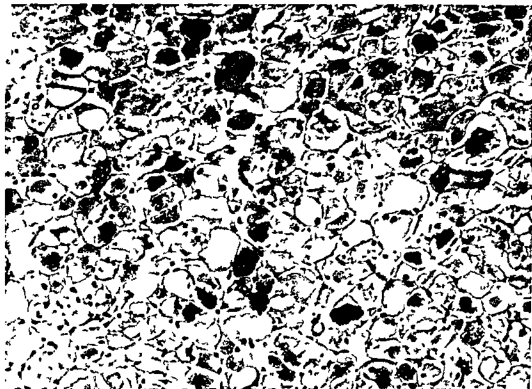
To date, 22 cases of extracardiac rhabdomyoma have been described in the literature (Assor & Thomas, 1965, Battifora et al, 1969, Czernobilsky et al 1968 De & Tribedi, 1940, Kay et al, 1969, Mikulowsky, 1972 Moran & Enterline, 1964, Occhinpti 1954 Pendl 1897, Rutz, 1926, Smith 1959, Tandler et al 1970, Tuazon [illegible]

We report here light and electron microscopic investigations of two further cases of rhabdomyoma both of which were localized in the submandibular region The characteristic histological picture of this tumour makes it easy to distinguish from other tumours occurring in this area

CASE HISTORIES

Case 1

A 62-year-old man was admitted on 19 XI 1969 to the ENT department, (no 09 02 09-0967) Hjorring Hospital, with a pigeon-egg sized palpable tumour of the right submandibular gland The size of the tumour had increased slowly over the course of 12 years and there had been no symptoms until the past few months, during which the patient had experienced pain and tenderness when eating The tumour was removed in its entirety on 20 XI 1969 The surgical specimen consisted of a submandibular gland measuring 35 × 20 × 20 mm, with concretions in the duct Microscopy revealed chronic/fibrotic sialoadenitis (due to sialolithiasis) There was nothing in the picture resembling rhabdomyoma At postoperative examination there was an indolent soft tissue swelling The patient was re-admitted to the department on 8 VI 1971 with a hen-egg sized intumescence in the upper part of the right side of the neck, lying in the submandibular region and extending into the lateral side of the neck The tumour had gradually increased in size since the operation in 1969 There were no symptoms On examination the tumour was soft, indolent, and freely mobile in relation to both skin and deep tissues There was no enlargement of the regional lymph



1 The tumour is built up of large, polygonal cells ı abundant cytoplasm with peripheral nuclei The arrangement of the tumour cells resembles that of striat ed muscle (case 1 haematoxyl n-eosin ×120)

nds Other clinical and laboratory investiga ns (including chest X ray, haemoglobin leu :yte and differential counts serum glutamine yacetic acid transaminase alkaline phospha- e se Na and se-K) were all normal Operation s carried out on 9 VI 1971 with removal of a ×40×30 mm reddish brown coarsely lobu ed well delimited tumour which lay deep to rnomastoid and extended from the upper ge of the thyroid cartilage to the angle of the v Deep to the upper part of the tumour, and arly separated from it there was a 6×5×3 n tumour of similar appearance This was o removed At follow up 11 months after eration there was no evidence of recurrence metastasis

se 2

29 year old Greenlander who had been ad itted to his local district hospital 13 years ously for tuberculosis and treated with chemotherapy for one year The patient was admitted on 2 XI 1971 to the Department of Medicine (no 13/72) Dronning Ingrid s Hos pital Godthaab, with a one year history of a swelling under the right mandible The patient was aware of the presence of the tumour, but otherwise had no complaints Examination revealed a pigeon egg sized indolent swelling immediately in front of and below the right angle of the jaw freely mobile in relation to the skin There was also a swelling in the floor of the mouth with displacement of the tongue towards the left The regional lymph glands were not enlarged Laboratory investigations (Hb, ESR) were normal X ray of the submandibular re gion revealed no evidence of salivary concretions X ray of the chest revealed evidence of old tuberculosis On the suspicion of tuberculosis of the submandibular gland the patient given anti tuberculous therapy, without ef Samples of the tumour were then taken



Fig 2 Tumour cells showing obvious striation in the cytoplasm and peripheral nuclei To the lower right of the picture slightly wavy, rod like crystalline structures can be seen (*arrow*) (case 2, phosphotungstic acid-hae toxylin, ×410)

scopy and for culture for tuberculosis bacillae After the histological report of the presence of a tumour the patient was transferred to the Department of Surgery At operation on 6 I 1972 a 30×20×20 mm, coarsely lobulated, reddish-brown, well-delimited tumour was removed Follow-up 7 months later revealed no evidence of recurrence or metastasis

MATERIAL AND METHODS

On removal the tumours were fixed in 4% formalin in isotonic phosphate buffer, pH 7 4, dehydrated, and embedded in paraffin 4–5 μm sections were stained with haematoxylin-eosin, Van Gieson–Hansen's stain, phosphotungstic acid haematoxylin (PTAH) and periodic acid Schiff (PAS), the latter with and without digestion with diastase In case 2, after a few days' fixation the tumour was divided into 5×2×2 mm pieces and transferred to Karnovsky's fi tion fluid (1965), diluted 1:1 with distilled wa

The pieces of tumour were post fixed in 1 osmium tetroxide cacodylate buffer, pH 7.5, hydrated first in ethyl alcohol and then propylene oxide, and embedded in Vestopal 1 μm sections were cut on an LKB Ultroto and stained with toluidine blue Ultrathin se tions were made from selected areas and sta ed with magnesium uranyl acetate (Frasca Parks, 1965) and lead acetate (Reynolds, 196 The slides were studied and photographed w a Siemen's Elmiskop I

Light microscopic investigations

The tumours, which were histologically iden cal, were built up of large, polygonal cells w abundant cytoplasm, shaped by mutual pre sure as there was little interstitial connecti tissue (Fig 1) The cytoplasm was eosinoph



g 3 Electron microscopic appearance of part of a abdomyoma cell There are widespread, branching yofibrils with a periodic arrangement of very thick Z-nds (*Z*), which occasionally lie singly (*arrow*) and na row pale I bands (*I*) There are many m some of which show well marked cristae (*C*) and ber of glycogen granules (*G*) (×40 000)

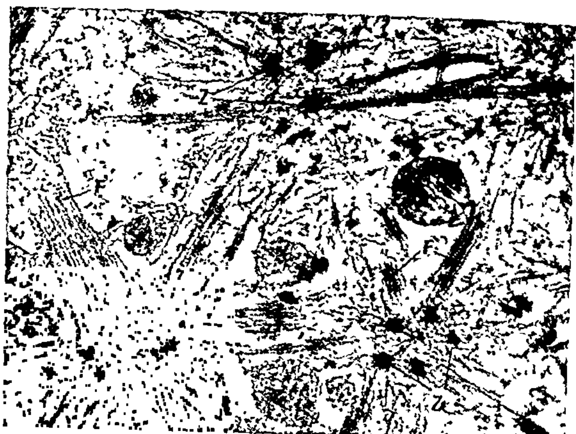


Fig 4 Electron microscopic apperance of myofibrils arranged in stellate figures with centrally placed, thick Z-bands (*Z*) From this the myofibrils (*F*) radiate out into the cytoplasm which contains a few mitochc (× 40 000)

and finely granulated, with numbers of vacuoles which were often situated in the cell periphery Some of the cells showed transverse striations (Fig 2), which were most obvious following staining with PTAH The nuclei often lay in the periphery of the cytoplasm, there was occasionally more than one, and there were obvious nucleoli There was no evidence of mitosis PAS-positive material could be demonstrated within the cytoplasm. PAS staining was negative following digestion with diastase In case 2 it was possible to demonstrate elongated, crystal-like structures in the cytoplasm of a few of the cells (Fig 2)

Electron microscopic investigations

In the immediate neighbourhood of the tumour there were fibrils of normal striated musculature The stroma was extremely scanty, with capillaries lined with endothelium The tumour was composed of cells delimited by an appare normal double-contoured plasma membran varying width There were numerous la round or oval mitochondria throughout cytoplasm These contained a moderately e tron-dense cotton-like matrix, although i few there were well marked cristae (Figs 3 a 4) Between these, in some cells, there w chaotically placed, frequently branching ru mentary myofibrils of varying length (Figs and 4) Each myofibril was composed of par lel filaments (Fig 3) The Z-bands seemed w prominent and often showed periodic arran ment, although they occasionally lay in iso tion In a few places there were A- and I bar (Fig 3) It was also possible to find a few st late figures composed of myofibrils which rad ated out from three or more Z-bands (Fig 4)

It was *not* possible to distinguish betwe thick and thin filaments in the individual my

ibrils Glycogen granules were seen in a number f places The sarcoplasmic reticulum was poorly eveloped and no triads composed of transverse ubuli and sarcoplasmatic reticulum were seen here were likewise no golgi complexes or larger olysomes The rod like crystalline structures hich were described in the histological preparations were not seen in the electron microscopic preparations

DISCUSSION

Ve consider that the two tumours presented ere represent benign neoplasms of the rhabdoyoma type, characterized by the well-marked elimitations of the tumour, the eosinophil ranular cytoplasm, the collections of glycogen nd not least, the striations

The region of predilection for extracardiac habdomyoma is the head and neck, 18 of the 2 cases reported hitherto being localized in this egion The remaining tumours were found in he axilla (De & Tribedi, 1940), the thoracic wall (Occhinpti, 1954) the stomach (Tuazon, 1969) and the labia majora (De & Tribedi, 1940) Two tumours, as in our case 1, were multifocal (Assor & Thomas, 1969, Goldmann, 1963) These tumours have been described in patients of all ages, from 8 months to 82 years of age, without predilection for any age group, there is likewise no difference in incidence in the two sexes In two cases the tumours were diagnosed shortly after birth both were localized to the tongue (Pendl, 1897, Rütz, 1926) In two other cases the tumour was first diagnosed at autopsy (Goldmann, 1963, Mikulowsky, 1972)

Symptoms and signs, which are completely non-specific, are exclusively dependent upon the localization and size of the tumour The sizes of those tumours reported in the literature ranged from 7 mm to 13 cm in diameter All tumours were well delimited, and could be removed radically There is no report of either recurrence or evidence of malignant transformation

Electron microscopic investigation of extracardiac rhabdomyoma (Battifora et al, 1969, Cornog & Gonatas, 1967, Kay et al, 1969, Tandler et al, 1970, Wyatt et al, 1970) reveals that the tumour reproduces the myofibril structure found in striated muscle but there is disturbance of the regular myofibril pattern Hypertrophic Z-bands, giving rise to thin filaments corresponding to the normal I filaments, were seen in all tumours (Czernobilsky et al, 1968) while in two there were also thick filaments corresponding to the normal A-filaments (Cornog & Gonatas, 1967, Tandler et al, 1970) In the tumour described in this report it was in some places possible to demonstrate both A- and I bands, implying the presence of both thin and thick filaments In one case (Tandler et al, 1970) all bands (Z, I, A-, H-, and M-bands) found in striated muscle have been reported, but their arrangement was random The varying findings on electron microscopic examination indicate that rhabdomyoma may show various degrees of myofibril differentiation The findings in embryonic muscle cells are similar, as thin filaments, are found earlier than thick filaments, and the arrangement is also random (Allen & Pepe, 1965)

In case 2 there was some degree of arrangement of the myofibrils, as in a number of places these were collected in stellate figures, this phenomenon has not previously been described, and can perhaps be interpreted as a tendency to a higher degree of organization

Crystal like structures, described here in case 2, have been described in a number of reports, and are considered to represent hypertrophied Z bands (Battifora et al, 1969, Czernobilsky et al, 1968, Kay et al, 1969, Smith, 1959, Tuazon, 1969) We have been unable to demonstrate this correlation between light and electron microscopic findings

As extracardiac rhabdomyoma occurs most commonly in the region of the head and it is necessary to bear this rare t in the differential diagnosis of region

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ZUSAMMENFASSUNG

Zwei Fälle eines Rhabdomyoms der Regio Submandibularis (der eine multifokal) werden beschrieben Elektronenmikroskopische Untersuchungen des einen Falls zeigten rudimentare Myofibrillen, teils unregelmässig, teils charakteristisch sternformig angeordnet Hypertrophische Z Bander und an einzelnen Stellen auch A- und I Bander konnten unterschieden werden Die Pathologie und die Klinik des Rhabdomyoms werden diskutiert Da extrakardiale Rhabdomyome am häufigsten in der Kopf Halsregion vorkommen, sollte dieser, obgleich sehr seltene Tumor in die Differentialdiagnose der Tumoren dieser Region mitgenommen werden

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